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ABSTRACT

This three-volume student text is designed for use by Air Force personnel enrolled in a self-study extension course for medical laboratory technicians. Covered in the individual volumes are hematology (the physiology of blood, complete blood counts and related studies, erythrocyte studies, leukocyte and thrombocyte maturation, and blood coagulation studies); laboratory procedures in blood banking and immunohematology (immunohematology, blood group systems, transfusion and transfusion practices, and the blood donor center); and serology (principles of immunology and serology; agglutination tests; latex-fixation, precipitin, and ASO tests; and serological tests for syphilis). Each volume in the set contains a series of lessons, exercises at the end of each lesson, a bibliography, and answers to the exercises. Supplementary volume review exercises and a change supplement are also provided. (MN)

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ED268334

MEDICAL LABORATORY TECHNICIAN--HEMATOLOGY, SEROLOGY, BLOOD BANKING AND IMMUNOHEMATOLOGY

(AFSC 90470)

EXTENSION COURSE INSTITUTE
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1. CHANGES FOR THE TEXT: VOLUME 1

a. Page 2, col 1, line 9 from bottom: Change "45,000 to "375,000" to "140,000 to 444,000."

b. Page 58, col 2, line 11: Change "12- x 75-mm" to "10- x 75-mm."

c. Page 107, answer 040-9: Change "12- x 75-mm" to "10- x 75-mm."

2. CHANGES FOR THE VOLUME REVIEW EXERCISE: VOLUME 1

a. Page 8, question 65, last line: Change choice "b" to "d."

b. Page 9, question 73: Change "(045)" to "(046)."

c. Page 12, question 105, choice a: Change "hyperplasia" to "hypoplasia."

d. Page 13, question 112: In the stem of the question, change "Breaker-Cronkite" to "Brecker-Cronkite."

e. Page 14, question 121: At the beginning of the stem of the question, insert "In the prothrombin consumption test."

f. The following questions are no longer scored and need not be answered: 1 and 74.

3. CHANGES FOR THE VOLUME REVIEW EXERCISE: VOLUME 2

a. Page 8, question 56: In the stem of the question, change "incomplete" to "incompatible."

b. Page 11, question 87: In the stem of the question, change "1° C. to 1° C." to "1° C. to 6° C."

c. The following questions are no longer scored and need not be answered: 64 and 79.

4. CHANGES FOR THE VOLUME REVIEW EXERCISE: VOLUME 3

a. Page 2, question 4: In the stem of the question, insert "certain" between "has" and "gram." Question 5: In the stem of the question, insert "lack the" between "mechanisms" and "specificity."

b. The following questions are no longer scored and need not be answered: 12 and 30.

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MEDICAL LABORATORY TECHNICIAN— HEMATOLOGY, SEROLOGY, BLOOD BANKING AND IMMUNOHEMATOLOGY

(AFSC 90470)

Volume 1

Hematology

Extension Course Institute

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THIS PUBLICATION HAS BEEN REVIEWED AND APPROVED BY COMPETENT PERSONNEL OF THE PREPARING COMMAND IN ACCORDANCE WITH CURRENT DIRECTIVES ON DOCTRINE, POLICY, ESSENTIALITY, PROPRIETY, AND QUALITY.

Preface

THIS COURSE, the final one in the Medical Laboratory Technician series, is made up of three volumes. These three volumes include hematology, blood banking, and serology. A glossary of technical terms used in Volumes 1, 2, and 3 of this CDC is printed at the back of Volume 3.

Chapter 1 of this volume provides a brief review on blood physiology. Chapter 2 discusses the complete blood count and related studies. Chapters 3 and 4 review more recent concepts in erythrocytes studies and leukocyte and thrombocyte maturation, with emphasis on application to the various classes of laboratories and their capabilities. Chapter 5, on blood coagulation studies, provides a new dimension in understanding the function and significance of coagulation factors, tests, and interpretation required to identify many coagulation problems.

Foldouts 1 and 2 are printed and bound in the back of this volume. Whenever you are referred to one of these foldouts in the text, please turn to the back of the volume and locate it.

Please note that in this volume we are using the singular pronoun *he, his,* or *him* in the generic sense, not the masculine sense. The word to which it refers is person.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to the School of Health Care Sciences/MSTW, Sheppard AFB TX 76311. NOTE: Do not use the suggestion program to submit corrections for typographical or other errors.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Behavioral Objective Exercises, Volume Review Exercise, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFS AL 36118, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 36 hours (12 points).

Material in this volume is technically accurate, adequate, and current as of April 1976.

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CHAPTER 1

NOTE: In this volume, the subject matter is developed by a series of Learning Objectives. Each of these carries a 3-digit number and is in boldface type. Each sets a learning goal for you. The text that follows the objective gives you the information you need to reach that goal. The exercises following the information give you a check on your achievement. When you complete them, see if your answers match those in the back of this volume. If your response to an exercise is incorrect, review the objective and its text.

The Physiology of Blood

THE WORD "HEMATOLOGY" is derived from the Greek words, *aima,* meaning blood, and *logos* meaning study. In this chapter, we will review the composition and functions of blood. Blood is a tissue in which the cells are suspended in a liquid medium. In this respect, it differs from other tissues of the body, which are also groups of specialized cells identified with a common function. The prime function of the blood is to maintain oxygen and food supply for the body cells and prevent accumulation of waste products. However, these are by no means the only functions of blood. Immunologic mechanisms, as well as other physical and chemical activities, involve the blood in many ways.

Since blood has a variety of complex functions, the composition of blood must also be complex. The average circulating total blood volume in a healthy male is 73 ml per kilogram of body weight and 63 ml per kilogram of body weight for healthy women. Formed elements account for nearly 45 percent of the blood volume; 90 percent of the remaining 55 percent is water, but the portion of liquid which is not water includes an uncalculated number of organic and inorganic materials.

1-1. The Composition of Blood

Our study of blood components will include both the cells and the medium in which they are suspended. We will consider the functions of blood in terms of the cellular and noncellular components. However, remember that separating the cells from the medium in which they are suspended is a privilege reserved for the laboratory technician. It is not realistic from a biological standpoint to completely separate these functions.

001. Match given blood components and related organs of blood formation with their appropriate descriptive statements.

Cellular Constituents. The well known cellular components of blood are the erythrocytes (RBCs) and the leukocytes (WBCs), as well as the platelets or thrombocytes. Where do these cellular elements come from and what are their functions? First, let's look at the RBCs.

Erythrocytes. The RBC is a living, metabolically active cell and not merely a small globule of protein which transports hemoglobin.

RBC development (erythropoiesis) is as follows: Primitive blood cells begin to form from specific tissue of the yolk sac early in the development of a human embryo. Following this period of development, the hepatic phase of blood cell development begins at about the second month of fetal life. Erythroblasts appear in the circulation as a result of development from mesenchyme cells in the liver tissue.

After erythropoiesis begins in the liver, the spleen produces erythrocytes to the end of the fourth month. The thymus is also active in producing blood cells, including RBCs, during this period of hepatic erythropoiesis.

During the last period of blood formation (hemopoiesis), the red bone marrow takes over the production of all blood cells. This period begins at about the fifth month. Unless the body is placed under some type of stress, the bone marrow alone produces both red and white blood cells. The primitive cells which gave rise to blood cells in fetal life revert to a "stand-by" status in the liver, spleen, lymph nodes, and other parts of the body, where they make up the so-called reticuloendothelial (RE) system. The RE system consists of a variety of different types of cells (histiocytes, Kupffer's cells, and others) whose function is to phagocytize particules such as bacteria or worn-out cells.

In the human being, the bone marrow normally produces red blood cells after birth. We have seen that erythropoietic activity in the fetus is quite different. Since it is generally the postnatal activity of erythropoiesis which is of greatest concern to the hematologist and to us, we should be interested in knowing how RBCs are produced in the marrow.

After birth, red blood cells are produced in the red marrow of the spongy bones. In the adult, this is restricted to the ribs, sternum, vertebrae, certain skull bones, and the epiphyses (ends) of the femur and humerus. This red marrow is in contrast to the yellow marrow which is contained in the diaphysis (shaft) of the long bones and has no erythropoietic activity. The red blood cells arise by cell division from *stem cells* in the red marrow. Stem cells are, by definition, undifferentiated cells which maintain their own numbers, but also give rise to other types of cells. Current theories hold that stem cells undergo two different kinds of division: the first to produce their own kind, and the second to produce at least one other type of cell. The stem cells may also produce their own kind of cell in this second type of division. The maturation sequence of erythrocytes is discussed in Chapter 3 of this volume.

It would be well to mention at this point that cell division in which the nucleus divides without a reduction in the number of chromosomes is referred to as *mitosis*. Blood cells are produced by mitosis of stem cells. This is unlike the reduction division (*meiosis*) which produces sex cells—that is, the sperm and the egg—in which there is a reduction in the number of chromosomes.

Leukocytes. WBCs of the types we find in adults are rarely found in the early states of fetal blood formation. Leukocyte production is generally believed to begin during the third or fourth month in the liver and continue to be produced there until a few weeks prior to birth. During this time, the thymus, lymph nodes, and spleen produce myelocytes and lymphocytes. After birth, lymphatic tissue plays a role in lymphocyte production, but the primary production is thought to be medullary (that is, it occurs in the bone marrow). Myelocytes and monocytes are definitely medullary in origin after birth. Researchers frequently distinguish between a small race of lymphocytes and larger lymphocytes with respect to life span, origin, and in some instances, with respect to function. We do not feel that this distinction is of any special importance to the medical laboratory technician. We will consider the maturation sequence of leukocytes in Chapter 4 of this volume. We generally divide leukocytes into granulocytes (neutrophils, eosinophils, and basophils) and agranulocytes (monocytes and lymphocytes).

Thrombocytes (platelets). Fragmented from the cytoplasm of megakaryocytes, thrombocytes vary considerably in size and shape. The cytoplasm contains a number of basophilic (purple) granules which can be observed on Wright stain smears. There are normally four to six thrombocytes per oil immersion field in areas of the slide where they are evenly dispersed. The normal platelet count as determined by phase microscopy is from approximately 45,000 to 375,000 per cubic millimeter.

Exercises (001):

Match the blood components and related organs of blood formation in column B with the appropriate descriptive statements in column A by placing the letter of the column B item beside the number of the column A item. Each column B item may be used once or more than once.

Column A

_____ 1. Are normally four to six per oil immersion field in areas of the slide where they are evenly dispersed.
_____ 2. The cell division in which the nucleus divides without a reduction in the number of chromosomes.
_____ 3. The reduction division which produces sex cells whereby there is a reduction in the number of chromosomes.
_____ 4. Plays an important role in lymphocyte production after birth.
_____ 5. Cells from which RBCs are formed by cell division.
_____ 6. Production in the bone marrow.
_____ 7. Primitive blood cells begin to form from specific tissue of the yolk sac.
_____ 8. Consists of a variety of different types of cells in spleen, liver, and lymph nodes, whose function is to phagocytize particles as bacteria or worn-out cells.
_____ 9. Term referring to RBC development.
_____ 10. Takes over the last period of blood formation.
_____ 11. After birth are produced in red marrow and spongy bones.
_____ 12. In the adult this activity is restricted to the ribs, sternum, vertebrae, certain skull bones, and the epiphyses of the femur and humerus.
_____ 13. Production believed to begin during the third or fourth month in the liver.
_____ 14. After birth, plays an important role in lumphocyte production.
_____ 15. Origin of myelocytes and monocytes.

Column B

a. Erythrocytes.
b. Leukocytes.
c. Thrombocytes.
d. Erythropoiesis.
e. Red bone marrow.
f. Reticuloendothelial system.
g. Stem cells.
h. Mitosis.
i. Meiosis.
j. Medullary (bone marrow).
k. Lymphatic tissue.

002. Indicate whether given statements correctly reflect the factors responsible for control of cell production, erythrocyte survival time and disposition, and some essential components of plasma and their significance.

Control of Cell Production. Evidence suggests that a well-balanced mechanism exists which maintains the *erythron* within normal limits and controls the response to a variety of normal and abnormal situations in the quantity of red blood cells. With respect to erythrocytes, the term "erythron" refers to precursor and mature cells as a single, though discontinuous, organ. Alterations in the concentration of hemoglobin in the blood lead to changes in tissue oxygen tension within the kidney. In response to hypoxia, the kidney secretes a factor that interacts with a plasma substrate to produce a hormone, erythropoietin. The major site of erythropoietin production is the kidney. This hormone induces primitive marrow cells to differentiate into pronormoblasts, subsequently causing the expansion of the erythroid marrow and an increase in red cell production. Erythropoietin is also related to tissue oxygen tension and is thought to be the most important factor controlling red cell production.

The control of leukocyte production is not as well understood as erythropoiesis. One reason for this is that red cells can be conveniently labeled by radioactive means and effectively traced. Further, the breakdown products of WBCs are not as readily observable, and RBCs do not continually move in and out of the blood as WBCs do.

The number of circulating cells, either white or red, is the net result of blood production minus destruction and blood loss. We have already mentioned some of the factors which control erythropoiesis. But how are worn-out cells disposed of? The normal RBC has been shown to have a *half-life* of 28 to 30 days when tagged with radioactive chromium (^{51}Cr). By half-life is meant the time required for one-half the number of erythrocytes tagged to disappear. Other methods yield somewhat different results. The average time of erythrocyte survival from the time the cells enter the peripheral blood until destruction is usually given as 120 days as determined by other methods. Increased destruction of erythrocytes is characteristic of hemolytic diseases. We will discuss erythrocyte abnormalities in greater detail in Chapter 3. Erythrocytes are normally disposed of by the reticuloendothelial system, particularly by the spleen. Hemoglobin that is released in red cell destruction is the source of bile pigments. Iron from blood destruction is used again in the production of new red blood cells.

Leukocytes enter and leave the peripheral blood throughout their life span. Consequently, the longevity of a leukocyte has not been worked out with certainty. In fact the fate of lymphocytes has not even been determined.

Plasma. That portion of blood which remains after the cells have been removed is, of course, plasma. From your study of clinical chemistry you will recall that plasma contains 3.6 to 5.6 grams of albumin per dl and 1.3 to 3.2 grams of globulin per dl. Plasma also contains fibrinogen, which is not present in serum. The level of fibrinogen in normal human plasma is 160–415 mg/dl. There are many other protein substances present, such as the coagulation factors.

In addition to proteins, plasma contains certain metabolic components required by cells. You are thoroughly familiar with many of these substances which are measured in the clinical laboratory. Glucose is probably the single most important food substance found in blood. Various ions present in plasma can also be considered nutrients for body cells as well as blood cells. All products of cells may also be found in the blood. In this category we find enzymes, antibodies, and hormones. Cellular waste products include urea, uric acid, and like compounds. In addition to dissolved solids, we find dissolved and combined gases. Foremost among these are oxygen, carbon dioxide, and nitrogen.

Exercises (002):

Indicate whether each of the following statements is true or false, and correct those that are false.

T F 1. Erythron refers to all mature erythrocytes as a single, though discontinuous organ.

T F 2. Alterations in the concentration of hemoglobin in the blood is inversely related to stability of tissue oxygen tension within the kidney.

T F 3. In response to hypoxia, the kidney secretes a factor that interacts with a plasma substrate to produce a hormone, erythropoietin.

T F 4. Erythropoietin is thought to be the most important factor controlling erythrocyte production.

T F 5. The control of leukocyte production is well understood and can be conveniently labeled by radioactive means and effectively traced.

T F 6. The number of circulating cells, either white or red, is the net result of blood production minus destruction and blood loss.

T F 7. The normal erythrocyte has been shown to have a half-life of 28 to 30 days when tagged with radioactive chromium (^{51}Cr).

T F 8. The time required for one-half the number of erythrocytes to disappear is termed half-life.

T F 9. Erythrocytes are normally disposed of by the reticuloendothelial system, particularly by the liver.

T F 10. Iron from blood destruction is not reused in the production of new blood cells.

T F 11. Leukocytes enter and leave the peripheral blood throughout their life span.

T F 12. The fibrinogen level in normal plasma is 0.160-0.415 mg/dl.

T F 13. As a single food substance, fat is probably the most important metabolic component in plasma.

1-2. Functions of Blood

In practicing hematology in the laboratory, we are concerned more with measurable evidence of dysfunction than with the final result of blood functions. We observe blood cells, measure chemical constituents, and perform serological tests to identify problem areas. This is the duty of a laboratory technician. We seldom reflect on the many complicated tasks the blood must perform.

You will note that we are describing functions of the blood with reference to other body tissues which the blood supports. We are not at this point especially concerned with the ways in which the blood maintains itself. If its only function were to sustain itself, there would be no justification for its existence. Yet, as technicians, we frequently think of blood dyscrasias as independent difficulties with the blood rather than with their effect on other organs of the body. This is because we are not involved in the clinical practice of medicine, but with scientific measurement of biological entities. As you progress to the 7 level, however, you must discern relationships as well as report single items of laboratory data. Please realize that you are not being asked to reach clinical conclusions, but to appreciate the involved interrelationships which exist among the mechanisms you are attempting to measure in the laboratory.

003. Point out the physiological process involved in the functions of the blood by defining external respiration, citing the gases involved and the average values, and by indicating the relationship between the various gas pressures both within the alveoli and in the bloodstream.

Oxygen-Carrying Capacity. The exchange of gases between the alveoli of the lungs and the bloodstream is referred to as *external respiration* and is pictured in figure 1-1. The exchange of gas between the blood and tissue cells is called *internal respiration*. Because respiration is a principal function of blood, we will now examine this process more closely. Please note that we are not using the term "respiration" in the popular context of "breathing." Thus, we will first discuss external respiration.

External Respiration. The gases with which we are concerned in the lungs, the blood, and the cells are nitrogen, oxygen, carbon dioxide, and water vapor. It is convenient to discuss these gases in terms of their pressures or tensions. By doing so, the gas exchange mechanism—pressure gradients—can be more easily understood. Average values of the pressures of these gases have been determined by experimentation, and it is most important to have a knowledge of these pressures in the four places mentioned in table 1-1. This table shows the average values in the alveoli, the arterial blood, the venous blood, and the tissue cells.

When air is breathed at a normal sea level pressure of 760 mm Hg, important changes begin to occur as the air enters the conducting passages and progresses toward the alveoli. Since the passageways contain water vapor at a pressure of 47 mm Hg, the air is diluted by the water vapor, and consequently the total air pressure is reduced by that amount (760 - 47 = 713). The moist air, now at a pressure of 713 mm Hg, enters the bronchial tree and then the alveoli. The alveoli, however, contain carbon dioxide at an average pressure of 40 mm Hg and nitrogen at its constant pressure of 573 mm Hg. The oxygen in the inspired air just prior to entering the alveoli has a pressure of approximately 150 mm Hg (21 percent of 713). The carbon dioxide present further penalizes this oxygen pressure until it has a value of 110 mm Hg. This value is called the "calculated" alveolar partial pressure of oxygen. It has been proven experimentally that this calculated value is slightly high. The alveolar partial

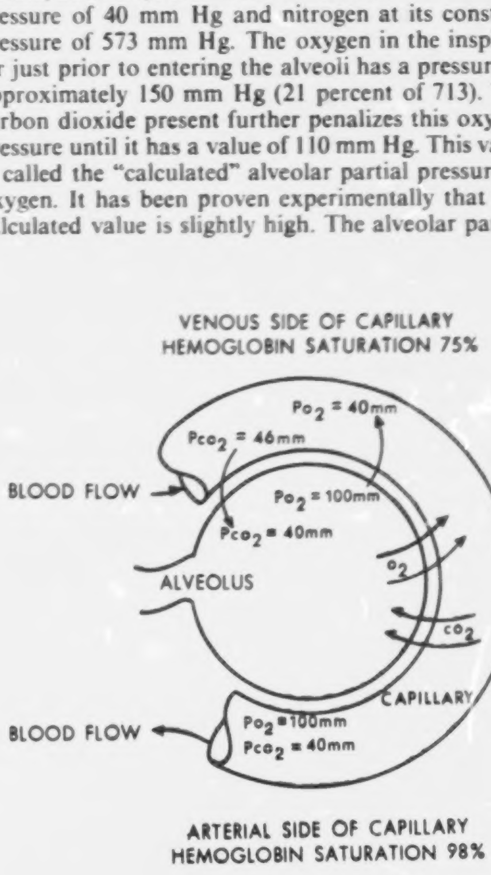


Figure 1-1. External respiration.

TABLE 1-1
AVERAGE VALUES OF GAS TENSIONS IN THE BODY

Gas	*Alveolus* mm Hg	*Arterial Blood* mm Hg	*Venous Blood* mm Hg	*Cell* mm Hg
Oxygen	100	100	40	1-60*
Nitrogen	573	573	573	573
CO_2	40	40	46	46
H_2O Vapor	47	47	47	47

Note: The value for nitrogen is constant; therefore, there is no exchange of this gas during the respiratory phases.

*Value controlled by tissue activity.

pressure of oxygen most frequently used is *100 mm Hg*. The slight difference is probably due to the fact that neither the atmospheric composition nor the action of carbon dioxide and oxygen in the alveoli is static, and also to other slight physiological variances.

If the inflation of the alveoli is considered to be static at a given point, figure 1-1 shows the relationship of the various gas pressures both within the alveoli and the bloodstream. The exchange of oxygen between the alveoli and the capillary is due to the great difference between the pressures. Since, according to the law of gaseous diffusion, gases will flow from a high-pressure area to one of lower pressure, oxygen diffuses through the walls of the alveoli and into the bloodstream. The pressure gradient is 60 mm Hg. Oxygen is constantly diffusing into the capillaries around the alveoli, and the exchange continues until a value of 100 mm Hg is reached within each capillary. At this point the hemoglobin of the red cells is saturated to approximately 98 percent of its capacity, as shown in figure 1-2. It should be noted that the oxygen pressure in the venous side of the capillary is 40 mm Hg. The percent saturation of the hemoglobin in this part of the capillary is, therefore, 75 percent (see fig. 1-1). This process is one of oxygenation (not oxidation).

At the same time that the exchange of oxygen is occurring, there is a similar transfer of the waste gas, carbon dioxide. The pressure of carbon dioxide in the venous blood is 46 mm Hg; in the alveoli it is 40 mm Hg. The exchange will proceed from the capillary blood into the alveoli. Although the carbon dioxide pressure gradient is only 6 mm Hg, a large quantity of gas flows across into the alveoli. This is explained by the fact that carbon dioxide has a greater coefficient of diffusion than does oxygen. As this exchange continues throughout the course of the capillary, the pressure of carbon dioxide in the blood falls until it reaches its normal value of 40 mm Hg, as noted in figure 1-1.

As the two gases flow in and out of the alveoli, the blood is oxygenated. The oxygen content is high and the carbon dioxide content is low. The blood then returns to the left side of the heart to be pumped throughout the body to all tissue cells. At this level, the second phase of respiration will occur—internal respiration.

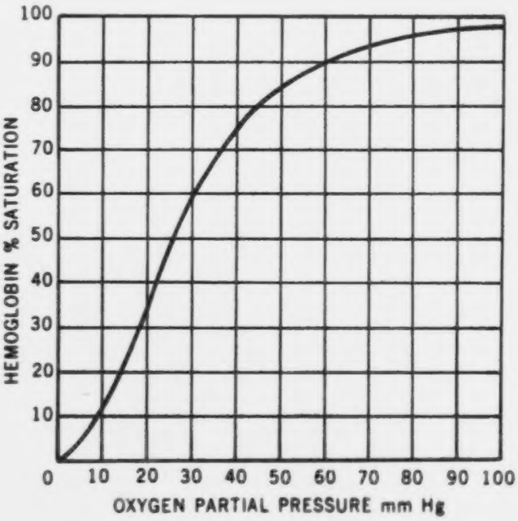


Figure 1-2. Dissociation curve of hemoglobin.

Exercises (003):

1. Define external respiration.

2. What are the gases of primary concern in the process of external respiration?

3. Since there is no exchange of nitrogen during the respiration phases, how is the value affected?

4. What effect does water vapor have on air pressure in the alveoli?

5. At what point will oxygen cease to diffuse into the capillaries surrounding the alveoli?

6. What is the physiological significance of the partial pressure of carbon dioxide in the venous blood as compared with the partial pressure of carbon dioxide in the alveoli?

7. How do you explain the fact that CO_2 diffuses readily between the venous blood and alveoli with a pressure gradient of only 6 mm Hg, whereas oxygen requires a much higher differential gradient to diffuse into the capillaries?

8. Since the passageways contain water vapor at a pressure of 47 mm Hg, to what amount of pressure is the moist air reduced?

9. Why is there a difference between the calculated alveolar partial pressure of oxygen and that of the value most frequently used?

In exercises 10-13, indicate whether each of the following statements is true or false, and correct those that are false.

T F 10. The exchange of oxygen between the alveoli and the capillaries is due to the great difference between the pressures.

T F 11. Since gases will flow from a low-pressure area to one of higher pressure, oxygen diffuses through the walls of the alveoli and into the bloodstream.

T F 12. When the oxygen partial pressure in the capillaries increases to 60 mm Hg, the hemoglobin in the red cells is saturated to approximately 20 percent.

T F 13. The partial pressure of carbon dioxide in the venous blood is 46 mm Hg; in the alveoli it is 40 mm Hg.

004. Point out the physiological process involved in the function of the blood by defining internal respiration, citing differences between arterial and venous blood, and by describing briefly a hemoglobin molecule.

Internal Respiration. We will now turn our attention to the exchange of gas between the blood and the tissue cells. The "pure" highly oxygenated blood with a low content of carbon dioxide leaves the left side of the heart with each contraction and travels throughout the arterial system to all of the capillaries.

The cells of all types of tissues are in contact with capillaries. As it enters the arterial side of the capillary, the arterial blood has gas tensions as follows: oxygen 100 mm Hg, carbon dioxide 40 mm Hg. These values are noted in figure 1-3. In the cell, oxidation is constantly occurring and, consequently, the oxygen content is at a low level. It varies in value, depending upon the activity of the cell at the time. A muscle cell, for example, during exercise would have a lower oxygen pressure than during a period of rest. The oxygen tension can vary from about 1 mm Hg to about 66 mm Hg. Since the oxygen pressure in the blood on the arterial side of the capillary is always higher than in the tissue cell, a large pressure gradient exists and there will again be a flow of oxygen to the lower pressure—in this case, from the blood into the cell. This transfer of oxygen is actually occurring throughout the length of the capillary until the pressure of oxygen in the blood gains equilibrium with the oxygen pressure in the cell.

While the transfer of oxygen is occurring, there is also a transfer of carbon dioxide. The constant metabolic activity of the cell results in a high production of carbon dioxide. The tension of carbon dioxide reaches 46 mm Hg. In the arterial blood in the capillary, the tension of carbon dioxide is about 40 mm Hg.

Even though a much smaller pressure gradient exists for this gas than for oxygen, a larger amount of carbon dioxide will be forced from the cell into the blood, due to the diffusion characteristics of carbon dioxide. As this transfer continues, the carbon dioxide tension in the blood increases to a level of about 46 mm Hg. Some of the carbon dioxide combines with hemoglobin and some is carried in the plasma. Thus, with the total exchange completed as the arterial blood passes the cell and enters the venous system, the blood will return to the heart to be pumped to the lungs via the pulmonary artery for another cycle of gaseous exchange. External respiration, as illustrated in figure 1-1, will then be repeated.

The structure of hemoglobin was discussed briefly in CDC 90411. You will recall that hemoglobin is a complex molecule with a molecular weight of 64.500 atomic mass units (amu). Four of its 10,000 atoms are iron, and each of these can carry 2 atoms of oxygen. Heme groups and chains of amino acids surround the four atoms of iron. The protein portion of the modecule consititutes the globin and functions in the transport of carbon dioxide.

There are many types of hemoglobin, some of which are characteristic of certain stages of life. We are born with a hemoglobin known as "F" for fetal. As a person matures, this changes to hemoglobin "A." This is the so-called normal form of hemoglobin. There are many abnormal forms of hemoglobin which can be identified by electrophoretic techniques. Best known of these are hemoglobin "C," which is associated with Mediterranean anemia, and hemoglobin "S," identified with sickle cell anemia.

Exercises (004):

1. What is meant by internal respiration?

2. What is the gas tension of oxygen and carbon dioxide in arterial blood?

3. What would be the oxygen pressure of a muscle cell during exercise (higher or lower)? Why?

4. What is the physiological difference between arterial and venous blood?

5. Do all arteries contain arterial blood in the physiological sense? Explain your answer.

6. Briefly describe the hemoglobin molecule.

005. Identify essential processes of erythrocyte metabolism.

Erythrocyte Metabolism. As we stated earlier, the red blood cell is not just a metabolically inert hemoglobin-carrying particle. On the contrary, the erythrocyte is a biologically active cell which itself respires, does work, and carries out biochemical reactions much the same way as any body cell. Energy is required to accomplish the active transport of glucose and ions against a gradient across the red cell membrane. The red cell must also supply energy in reducing and converting iron to the ferrous state. As hemoglobin oxidizes, it shifts to the ferric state, thus converting to methemoglobin. The shift and the reverse process may be diagrammed as follows:

Reduced Hemoglobin		Oxyhemoglobin (Methemoglobin)
FERROUS STATE	ENERGY	FERRIC STATE
Fe++	$\leftrightarrows$ $+O_2$	Fe+++

Most of the red blood cell consists of hemoglobin. Yet the RBC does contain a number of other substances, and a deficiency or absence of one or more of

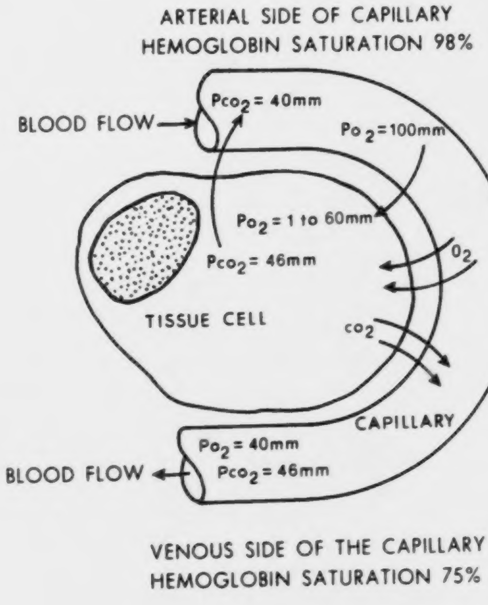


Figure 1-3. Internal respiration.

these substances can result in anemia. Many enzymes have been identified from studies of the erythrocyte. For example, the effect of glucose-6-phosphate dehydrogenase deficiency was explained in CDC 90411.

Exercises (005):

1. How does the hemoglobin molecule "carry" oxygen?

2. What is required to accomplish the active transport of glucose and ions against a gradient across the red cell membrane?

006. Identify related terms and the functions of the different types of leukocytes, and point out some constituents and functions of platelets.

Leukocyte Functions. Leukocytes remove invading antigens (for example, bacteria) and to some extent transport and distribute antibodies. Monocytes and all of the granulocytes have been shown to demonstrate directional movement. Their movement is subject to *chemotaxis,* which is defined as the response of living protoplasm to a chemical stimulus. This is a means of attracting cells to substances which they must either transport or engulf. The process of engulfing and destroying bacteria, or *phagocytosis,* is a prime function of leukocytes.

Monocytes. These cells will engulf bacteria and larger materials, including even protozoa and red cells, and are called *macrophages.* In this regard, monocytes are perhaps the most efficient phagocytes of all the cells. Monocytes contain many of the lytic enzymes that are found in microphages (granulocytes).

Neutrophils. Neutrophilic leukocytes are excellent *microphages.* That is, they engulf bacteria and other microscopic particles. The particles are first surrounded by cellular pseudopodia and then incorporated into a cell vacuole. There the foreign bodies mix with substances released from the cytoplasm of leukocytes. In this way the leukocyte is not injured by whatever "combat activity" is taking place in the vacuole. Eventually the granulocytes disintegrate and in inflammatory processes are succeeded by monocytes. In addition, monocytes contain lipases which enable them to dissolve the lipoid capsules of certain bacteria. Neutrophils are fully developed (mature) cells that are incapable of mitotic division. They carry on active metabolism.

Eosinophils. As you already know, eosinophils are found in tissue fluid as well as in peripheral blood, especially in areas where there is an allergic reaction. Current thinking holds that eosinophils are involved in the antigen-antibody reaction. They have been shown to phagocytize antigen-antibody reactants. Eosinophils are also thought to transport, or at least contain, lysins which act on fibrin. It is suggested that eosinophils limit the action of substances such as histamine. How this is accomplished is not yet clear. The mobilization of eosinophils from their reserve in the bone marrow is at least in part under hormonal control. If the adrenal cortex is functioning properly, an injection of adrenocorticotropic hormone (ACTH) results in a marked decrease in the number of circulating eosinophils and in the number of circulating lymphocytes. On the other hand, there is an increase in the number of circulating neutrophils.

Basophils. The function of basophils in man has not been ascertained. They quite possibly represent a vestige of evolution. Their granules have been found to contain heparin, and these cells frequently appear during the clot dissolution phase of an injury. Hence, it has been suggested they may be involved in clot absorption.

Lymphocytes. The lymphocyte is now believed to be directly connected with antibody production. Undoubtedly, the lymphocyte performs important immunolgic functions. According to very recent studies, many of the activities previously thought to take place in the reticuloendothelial (RE) system actually take place in lymphocyte tissue.

Platelets. Platelets possess metabolic systems, expend energy, and respond to stimuli. They contain many enzymes and undergo respiratory activity and glycolysis. They possess coagulation factors usually designated as PF-1, PF-2, and on through PF-9 (at the present time). The cells contain fibrinogen and vasoconstrictor substances, calcium, and many other components which are either known or presumed to participate in the clotting mechanism. Clot-promoting lipoproteins are also found in platelets. In addition, well-defined antigens have been found in platelets. This partially explains the occurrence of incompatibility reactions when platelets are infused into the blood during a transfusion. In Chapter 5 of this volume, we will consider the role of platelets in the blood coagulation mechanism.

Exercises (006):

Match the terms, leukocytes, and platelets in column B with the definitions/descriptions to which they are closely related in column A by placing the letter of the column B item beside the number of the column A item. Each column B item may be used once, more than once, or not at all.

	Column A	*Column B*
______ 1.	The response of living protoplasm to chemical stimulus.	a. Eosinophils. b. Basophils.
______ 2.	The process of destroying and engulfing bacteria, a prime function of leukocytes.	c. Lymphocytes. d. Platelets. e. Monocytes.

______ 3. These cells will engulf bacteria and materials including even protozoa and red cells.
______ 4. Contain many of the lytic enzymes formed in granulocytes.
______ 5. Are excellent microphages.
______ 6. Cells by which the inflammatory process is succeeded after the disintegration of granulocytes.
______ 7. Contain lipases which enable them to dissolve the lipoid capsules of certain bacteria.
______ 8. Are found especially in areas where there is an allergic reaction in tissues or peripheral blood.
______ 9. If the adrenal cortex is functioning properly, an injection of adrenocorticotropic hormone (ACTH) results in a marked decrease in the number of these cells.
______ 10. Their granules have been found to contain heparin, and they frequently appear during the clot dissolution phase of an injury.
______ 11. Directly connected with antibody production.
______ 12. They process coagulation factors usually designated as PF-1 through PF-9.
______ 13. Cells contain fibrinogen, vasoconstrictor substances, and calcium.
______ 14. Contain clot-promoting lipoproteins.

f. Chemotaxis.
g. Macrophages.
h. Phagocytosis.
i. Microphages.
j. Neutrophils.

007. Point out the muscles of the heart and other organs and their functions along the path of the pulmonary and systemic circulation.

Circulation. Blood can perform its functions only if it reaches all body tissues and follows a regular circulatory pattern which permits oxygenation and removal of waste products. The circulation of blood depends upon several factors. Most important are the rhythmic beating of the heart, the volume of blood circulated, the condition of the blood vessels, and a system of valves to control the direction of flow.

The heart causes blood to circulate in this way: When the muscles of the atria contract, blood is forced into both ventricles simultaneously. The wave of contraction then passes to the ventricles, which also contract and force the blood out into the arteries. Thus, blood is pumped throughout the entire body. Blood is circulated through both *pulmonary* and *systemic* circulation. As you read the descriptions, trace the course of blood in figure 1-4.

Pulmonary circulation. Pulmonary circulation, sometimes calles lesser circulation, is the circulation of blood through the lungs for the purpose of oxygenation. All venous blood returning from the body enters the right atrium through the superior vena cava or the inferior vena cava and then enters the right ventricle through the atrioventricular (tricuspid) valve. As the right ventricle contracts, venous blood is forced through the pulmonary valve to the pulmonary artery, which carries the blood to the lungs, where the blood, through diffusion, exchanges waste carbon dioxide for oxygen. The richly oxygenated blood is then returned to the left atrium via the pulmonary veins.

Systemic circulation. Systemic circulation involves the circulation of blood from the left ventricle to all parts of the body and then back to the right atrium. When the left ventricle contracts, blood is forced through the aortic valve into the aorta. From here it takes different courses to all parts of the body through arteries, arterioles, and capillaries and then returns by way of the veins to the right atrium. Systemic circulation includes *coronary* and *portal* circulation.

a. Coronary circulation involves the circulation of blood through the muscular tissues of the heart.

b. Portal circulation involves the passage of venous blood from the gastrointestinal tract and spleen, through the liver, and out to the inferior vena cava through the hepatic veins.

Exercises (007):

1. Blood is forced into both ventricles when the muscles of the ______________ contract.
2. The wave of contraction then passes to the ventricles, and the blood is forced out into the ______________________ .
3. Pulmonary circulation is the circulation of the blood through the lungs for the purpose of ______________________ .
4. All venous blood returning from the body enters the ______________ atrium through the ______________ or the ____________ .
5. The blood then enters the right ventricle through the ______________________ valve.
6. The right ventricle contracts, and venous blood is forced through the ______________ to the ____________ ____________ , which carries the blood to the lungs.
7. In the systemic circulation, richly oxygenated blood is returned to the left ____________ via the pulmonary ______________ .
8. The blood enters the left ventricle through the ______________________ valve.
9. When the left ventricle contracts, the blood is forced through the ______________ valve into the ______________________ .
10. Systemic circulation includes ______________ and ______________________ circulation.
11. ______________ circulation involves the circulation of blood through the muscular tissues of the heart.
12. ______________ circulation involves the passage of venous blood from the gastrointestinal tract and spleen, through the liver, and out to the other inferior vena cava through the hepatic veins.

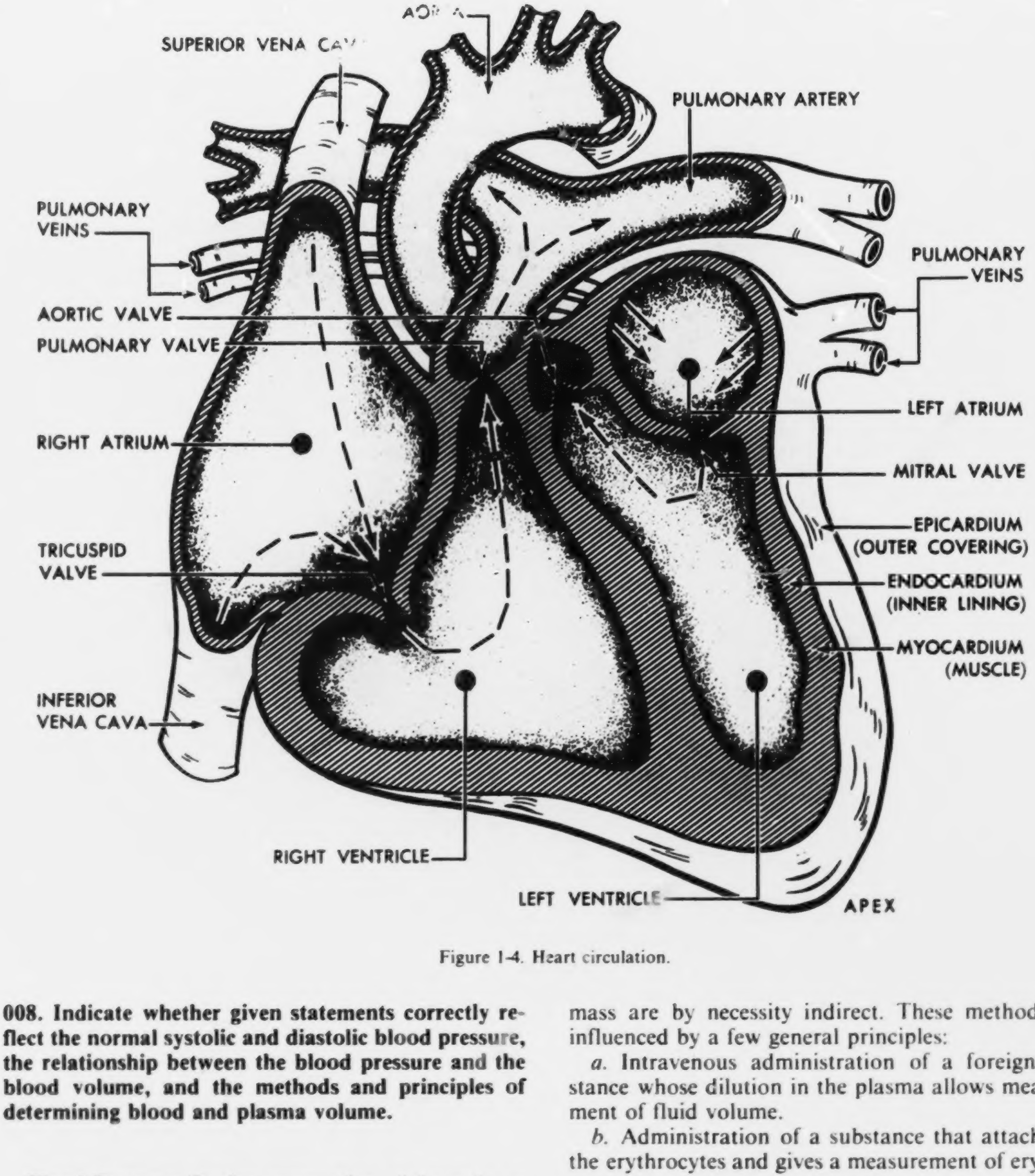

AORTA
SUPERIOR VENA CAVA
PULMONARY ARTERY
PULMONARY VEINS
PULMONARY VEINS
AORTIC VALVE
PULMONARY VALVE
LEFT ATRIUM
RIGHT ATRIUM
MITRAL VALVE
EPICARDIUM (OUTER COVERING)
TRICUSPID VALVE
ENDOCARDIUM (INNER LINING)
MYOCARDIUM (MUSCLE)
INFERIOR VENA CAVA
RIGHT VENTRICLE
LEFT VENTRICLE
APEX

Figure 1-4. Heart circulation.

008. Indicate whether given statements correctly reflect the normal systolic and diastolic blood pressure, the relationship between the blood pressure and the blood volume, and the methods and principles of determining blood and plasma volume.

Blood Pressure. During contraction of the atria, or atrial systole, blood is forced through the atrioventricular valves into the ventricles. The resulting pressure is termed systolic pressure. In a normal adult this systolic pressure as measured in the bronchial artery ranges from 90 to 140 mm Hg. Atrial systole is followed by atrial diastole in which blood flows into the empty atria. The normal range for diastolic pressure in the adult is 60 to 90 mm Hg. Blood pressure depends upon blood volume. As volume is diminished, the blood pressure falls unless there is a compensating decrease in the lumen of the blood vessels.

Blood and Plasma Volume. Methods used to measure blood volume, plasma volume, and total RBC mass are by necessity indirect. These methods are influenced by a few general principles:

a. Intravenous administration of a foreign substance whose dilution in the plasma allows measurement of fluid volume.

b. Administration of a substance that attaches to the erythrocytes and gives a measurement of erythrocyte mass.

c. Calculation of the other compartment from data obtained by methods *a* or *b* plus the hematocrit value.

d. Simultaneous but independent measurement of plasma volume and RBC mass.

Earlier methods such as the Evans blue dye method for plasma volume and the ^{51}Cr method for RBC mass have been largely replaced by the simultaneous method that is not dependent on the hematocrit. One technical difficulty with methods used when the foreign substance is expected is the significant diffusion into extravascular spaces. This occurs with Evans blue and ^{131}I-tagged albumin. Impaired circulation will modify the time required for complete mixing of the administered substance with the blood. A new method based on tagging the hemoglobin with "C" will probably minimize extravascular diffusion when isotopes with an affinity for erythrocytes are used. However, this method is still in the developmental stage.

The recommended method is the simultaneous measurement of plasma volume with ^{131}I-albumin or ^{125}I-albumin and RBC mass with ^{51}Cr. Total blood volume is the sum of the two measurements.

Venous, Arterial Blood, and Concentration. As noted from the physiological changes encountered during circulation, it should also be apparent to you that venous blood differs somewhat from arterial blood. For example, the CO_2 differs. Recent studies have shown that blood taken from a properly performed "finger stick" does not differ significantly in most routine hematologic values (that is, hematocrit) from venous blood. This is because blood that is obtained from a cutaneous fingertip puncture comes mainly from metarterioles and venules.

Hemoglobin values alone can often be misleading. Suppose a patient suddenly begins to bleed profusely. The blood volume would drop, and the patient's blood pressure would also drop. Until tissue fluids move into the vascular system, or until the patient receives fluids intravenously, the hemoglobin and hematocrit will not *immediately* change. This is because the results of these tests are reported in terms of *concentration* rather than total blood volume. While the concentration of cells hasn't changed significantly, the total number may be markedly changed.

This can be seen in the following example. A patient loses 2 pints of blood due to an acute injury. The 2 pints of blood contain both a certain number of cells (concentration) as well as a certain amount of plasma (volume). If we perform a hemoglobin on the patient and repeat the test 30 minutes later, the test results will likely be similar. This happens because the amount of hemoglobin in a unit of plasma has not changed, since both cells and plasma were lost at the same time. Later, when the body replaces the lost blood volume, the concentration of hemoglobin will decrease. Variations in concentration/blood volume are sometimes due to deviation in the testing procedure and may well be within acceptable limits.

Exercises (008):

Indicate whether each of the following statements is true or false, and correct those that are false.

T F 1. In the normal adult the systolic pressure as measured in the bronchial artery is 60 to 90 mm Hg.

T F 2. The normal range for diastolic pressure is 90 to 140 mm Hg.

R F 3. As blood volume diminishes, the blood pressure falls unless there is a compensating decrease in the lumen of the blood vessels.

T F 4. One of the principles influencing the methods used to measure blood volume is based upon the administration of a substance that attaches to the erythrocytes and gives a measurement of erythrocyte mass.

T F 5. Evans blue dye method for plasma volume and the ^{51}Cr method for RBC mass are currently used for measuring blood volume over the simultaneous method not dependent on the hematocrit.

T F 6. One technical difficulty with the Evans blue and ^{131}I-albumin is the significant diffusion into extravascular spaces.

T F 7. The recommended method is the simultaneous method of plasma volume with ^{131}I-albumin or ^{125}I-albumin and RBC mass with ^{51}Cr.

T F 8. Recent studies have shown that the blood taken from a properly performed "finger stick" differs significantly from venous blood.

T F 9. When a patient suddenly begins to bleed profusely, the hematocrit and hemoglobin will change immediately.

T F 10. Variations in concentration/blood volume are sometimes due to deviation in the testing procedures and may well be within acceptable limits.

CHAPTER 2

The Complete Blood Count and Related Studies

MOST WORK in hematology consists of complete blood counts (CBCs). In view of this, the technician must master procedures inherent in a CBC and know the normal values. The complete blood count consists of five tests: the red cell count, the white cell count, hemoglobin, hematocrit, and the differential white cell count.

Blood can be drawn by venous or capillary puncture. The choice of specimen is often determined by the amount of blood needed to do the tests ordered by the physician, as well as by the age and condition of the patient. Generally, venous samples are preferred for laboratory analysis, since they provide enough blood to perform multiple laboratory procedures or to repeat procedures for accuracy. However, capillary specimens are quickly obtained without disturbing a vein which should be kept free of scars for a potentially greater need. If you use capillary blood, it is important to have a free-flowing puncture. Capillary specimens, taken properly, will give hematology results comparable with venous specimens.

2-1. Collecting a Blood Sample

We will discuss aspects of getting a good sample, preserving it, and doing the tests correctly. It is always worthwhile to reaffirm good technique, since one can easily develop undesirable habits when performing simple procedures. This is especially true when the workload is heavy. Prominent qualities of a good blood collector are skill, patience, and understanding.

009. Indicate whether given statements correctly reflect the techniques and sites for capillary puncture.

Capillary Puncture. Capillary puncture is a common method of collecting a blood sample. *If correctly performed,* it is easy and simple, and causes less pain and anxiety to the patient than other methods. Blood cell distribution is the same as that normally found in venous blood. A capillary blood sample is not usually treated with anticoagulant (except in the microhematocrit), and this lessens the possibility of cell distortion.

Several different sites are suitable for capillary puncture. The lateral surface just beyond the distal joint of a finger is the best site. The center part of the finger is most sensitive, and for this reason should be avoided. Heavily calloused areas or areas with excessive tissue fluids (edema) result in poor samples. The lobe of the ear may also be used for capillary puncture. Differences in cell concentration do occur when blood is obtained from this site, primarily because of a high lymphocyte concentration in the ear lobe. And too, it is not convenient to work with a patient's ear. Because of the small size of the finger, in an infant the preferred puncture site is the heel or big toe. A modification of the normal technique that has proven quite satisfactory when working with infants is to make two incisions in a criss-cross fashion or "T" if a fairly large quantity of blood is required. The Bard-Parker blade is effective, although its use is somewhat dangerous because of the nearness of tendons found in the heel region. It is probably better to use an ordinary hemolet unless you are performing, in addition to a CBC, microchemistry studies or other procedures that require more blood. Adequate medical supervision and training is a prerequisite to use of the "T" incision. Remember, capillary blood must be collected from a *free-flowing* puncture wound; otherwise, the laboratory results will not be valid.

Exercises (009):

Indicate whether each of the following statements is true or false, and correct those that are false.

T F 1. Blood cell distribution from a capillary puncture is slightly different from that found in venous blood.

T F 2. The lateral surface just beyond the distal joint of a finger is the best site.

T F 3. Heavily calloused areas or areas with excessive tissue fluids (edema) result in the best samples.

T F 4. Differences in cell concentration do occur when blood is obtained from the ear lobe, primarily neutrophils.

T F 5. In a modification of the normal technique, two incisions in a criss-cross fashion have proven quite satisfactory.

T F 6. The use of the Bard-Parker blade is effective for making the criss-cross incisions and presents a lesser chance than the ordinary hemolet of cutting a tendon in the infant's heel.

010. Point out techniques for venipuncture and precautions before, during, and after blood collection, and state the given time in which blood counts should be done after collection.

Venipuncture. The first step in performing a venipuncture is selection of the best vein. Select a vein that is large, readily accessible, and sufficiently close to the surface to be seen or palpated. Do not guess at the location of the vein or attempt to find it by probing. In a clean, atraumatic venipuncture, the needle goes directly into the vein. There is no probing or tearing of tissue. Atraumatic technique is necessary to obtain a suitable specimen as well as for patient comfort. If you have difficulty, do not hesitate to seek help. Even the most experienced technician will have difficulty once in a while, whereas another technician may find the vein on a particular patient without trouble.

It is important that correct technique be used to avoid unnecessary pain to the patient and prevent tissue damage. It is also imperative to secure a good blood specimen and to prevent contaminating the specimen or infecting the patient. A cardinal rule in the practice of medicine for which you fulfill a supporting role is to avoid harm to the patient. Veins may become distended and easier to enter by allowing the arm to hang down for 2 or 3 minutes, by massaging the blood vessel toward the body, or by gently slapping the site of puncture. Young and vigorous persons usually have elastic veins well filled with blood. Elderly or debilitated persons may have sclerosed or fragile veins, which are hard to enter or which collapse easily.

If venipuncture is a problem due to age of the patient, scarring from repeated venipuncture, or any other unusual circumstance, the technician should obtain assistance from a more experienced technician or consult a physician. *Under no circumstances should a technician draw blood from a jugular vein or femoral vein.* When venipuncture is used to obtain blood for quantitative analyses or coagulation studies, the blood must be obtained rapidly from an area that has good circulation. If the tourniquet is kept in place for longer than 2 minutes, changes may occur in the concentration of blood components. The blood should be aspirated into a clean, dry syringe without delay. In some cases (for example, the Lee White clotting time), siliconized syringes or syringes rinsed in normal saline are recommended.

Occasionally, the best vein is found on the hand, leg, or foot. These areas are more sensitive, however, and the veins are not as firmly anchored as those of the arm. Rolling veins may be held firm by placing the thumb on the vein so that a 1 or 2 inch length of vein lies between the thumb and the puncture site.

To do a venipuncture, you also need a cleansing agent. The most common cleansing agent used to clean the skin areas over the site of venipuncture is 70 percent isopropyl alcohol. Keep in mind that alcohol does not sterilize and, in fact, is not usually sterile itself. Neither is alcohol a very effective antiseptic, but it does remove the film of natural oils and tissue debris. A precaution should be taken not to use alcohol sponges that are too wet; that is, excess alcohol (along with debris from hands) should not be rinsed back into the container.

If it is difficult to enter the vein, or if a hematoma forms, release the tourniquet, promptly withdraw the needle, and apply pressure to the puncture site. Do not reapply the tourniquet to the same arm for at least 20 minutes. Vigorous pulling on the plunger of the syringe may collapse the vein, produce hemolysis of the blood specimen, or cause air to enter the syringe. When repeated venipunctures have to be done on one patient, select different sites for blood withdrawal. If the vacutainer assembly is being used, as soon as the needle is in the vein, push the tube firmly but carefully in as far as it will go, insuring that the needle is kept steady. If a patient has intravenous solutions going into both arms, it is acceptable to puncture the veins 3 or 4 inches below the site of the I.V.

When you have finished the venipuncture, insure that pressure is maintained on the site a full 3 to 5 minutes. Be sure the sleeve of the patient's shirt or other garment is not too tight, for this can act like a tourniquet. It is helpful for the patient to hold his arm upward as if he were reaching toward the ceiling. The common technique of merely bending the elbow after venipuncture is acceptable. However, if the elbow is bent too forcefully, the clothing may bind the vein and restrict venous flow. Further, raising the arm upward will slow blood flow and minimize leakage at the puncture site.

Blood drawn by venipuncture is often stored for a period of time before it is analyzed. For this reason, you should take the following precautions to insure a valid analysis. Before withdrawing blood from its container, mix the blood sample thoroughly but gently. Blood tubes should be tightly stoppered at all times to prevent contamination and possibly even evaporation. Store the blood specimen in a refrigerator, with the exception of serum for cold agglutinins, which should be separated from the clot before low temperature storage. Blood counts should be done within 3 hours after collection, and specimens taken for a CBC should never be stored overnight.

Exercises (010):

1. When obtaining blood by venipuncture, what cardinal rule in the practice of medicine must the technician follow?

2. Initially, what may be done with the arm to make it easier to enter the veins?

3. What should the technician do if venipuncture is a problem due to age of the patient, scarring from repeated venipuncture, or any unusual circumstance?

4. What technique should a technician use to obtain blood from a jugular vein or femoral vein?

5. What condition will result if the tourniquet is kept in place for longer than 2 minutes?

6. How can one obtain blood from rolling veins?

7. What should be done if it is difficult to enter the vein, or if a hematoma forms?

8. If a patient has intravenous solutions going into both arms, how is it acceptable to obtain blood?

9. Why is it helpful for the patient to hold his arm upward as if he were reaching for the ceiling after venipuncture?

10. How soon after collections should blood counts be done?

011. Identify the anticoagulant of choice for complete blood counts in terms of application, advantages, and disadvantages.

Anticoagulants. Many laboratory procedures must be performed on whole blood or plasma. Thus, after the blood is obtained from the patient, it is mixed with an anticoagulant to prevent coagulation. The choice of anticoagulant will depend on the analysis to be made. We will discuss five anticoagulants commonly used for hematology procedures.

Ammonium and potassium oxalate (Heller and Paul double oxalate). This anticoagulant consists of six parts of ammonium oxalate and four parts of potassium oxalate. The concentration used is 2 mg per milliter of whole blood. Coagulation is prevented through formation of an insoluble calcium oxalate. There is one disadvantage to using Heller and Paul double oxalate for the preparation of blood films. A few minutes after the blood is mixed with this anticoagulant, crenation of the red blood cells, vacuoles in the granulocytes, and bizarre forms of lymphocytes and monocytes will occur.

Heparin. Heparin may be used in a concentration of 0.2 ml of saturated heparin per 1.0 ml of whole blood. Coagulation is prevented for a period of approximately 24 hours by the neutralization of thrombin. When anticoagulated blood is used for the osmotic fragility test, heparin is the anticoagulant of choice. On the other hand, heparinized blood has been unsatisfactory for differential smears which are to be Wright-stained, due to the formation of a blue background on the stained smears.

EDTA (sequestrene or versene). EDTA is the disodium or dipotassium salt of ethylenediaminetetra-acetic acid. EDTA is excellent for prevention of platelet clumping. EDTA is the anticoagulant of choice for a CBC. This anticoagulant causes a minimum of distortion to the cells and platelets. It does not dissolve quickly in blood, however, so the tube must be inverted four or five times when blood is added. The dipotassium salt is prepared as a 1 percent solution in distilled water, and a final concentration of 0.5 ml of anticoagulants for each 5 ml blood is used.

Coagulation is prevented by the removal of calcium from the blood by precipitation or binding in unionized form. Formation of artifacts is prevented, and it may be used for preparation of blood smears up to 2 hours after blood collection. Erroneous values of the hematocrit and sedimentation rate will be obtained at concentrations greater than 2 mg per ml of whole blood. The hemoglobin will be unaffected. When stored at 4° C for 24 hours, blood may be used without any effect shown in the hemoglobin, hematocrit, white blood count, or red blood count. Blood stored at room temperature will show an elevated hematocrit reading; however, the remaining tests, except the ESR, will not be affected.

Sodium citrate. Sodium citrate is the anticoagulant of choice for coagulation studies. The concentration used is one part 0.11 M sodium citrate to nine parts of whole blood. It prevents coagulation by binding the calcium of the blood in a soluble complex and protects certain of the procoagulants.

Sodium oxalate. Sodium oxalate is a widely used anticoagulant in coagulation studies. The concentration used is one part 0.1 M sodium oxalate to nine parts of whole blood. In preventing coagulation, the sodium oxalate combines with calcium in the blood to form insoluble calcium oxalate.

Exercises (011):

Match each anticoagulant in column B with the appropriate descriptive statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

_____ 1. Coagulation is prevented by the removal of calcium from whole blood.

_____ 2. After a few minutes, mixing will cause crenation of RBCs, vacuoles in the granulocytes, and bizarre forms of lymphocytes and monocytes.

_____ 3. Coagulation is prevented for a period of approximately 24 hours by the neutralization of thrombin.

_____ 4. Used in a concentration of 0.2 ml of saturated solution per 1.0 ml of whole blood.

_____ 5. Used in a concentration 0.5 ml of anticoagulant for each 5 ml of blood.

_____ 6. The anticoagulant of choice for osmotic fragility.

_____ 7. Erroneous values of the hematocrit and sedimentation rate will be obtained at a concentration greater than 2 mg per milliliter of whole blood.

_____ 8. The anticoagulant of choice for coagulation studies.

_____ 9. Prevents coagulation by combining with calcium in the blood to form insoluble calcium compound.

_____ 10. Prevents coagulation by binding the calcium of the blood in a soluble complex, and protects certain of the procoagulants.

_____ 11. Blood may be stored at 4° C without any effect shown in the hemoglobin, hematocrit, white blood count, or red blood count.

_____ 12. Blood stored at room temperature will show an elevated hematocrit; other remaining tests will not be affected.

Column B

a. Sodium oxalate.
b. Heparin.
c. EDTA.
d. Sodium citrate.
e. Ammonium and potassium oxalate.

2-2. Cell Counts

The process of counting cells is a significant chore in any laboratory. In this section we will discuss red blood cell counts (RBC), white blood cell counts (WBC), cerebrospinal fluid cell counts (CSF), and counts of spermatozoa in semen. We will also consider automated cell counting and some of the quality control measures that will help you achieve accurate counts.

012. Point out techniques, dilution factors, and sources of error in performing the manual red blood cell count.

Red Blood Cell Count. Unless the laboratory is using more sophisticated counting methods such as the Model S Coulter Counter® or the Hemac Laser Hematology Counter, the red blood cell count is not included routinely in the complete blood count. When performing a manual red blood cell count, use two separate pipettes and fill two chambers from each pipette. The average count is used to calculate blood indices.

When a venous sample is used, mix it thoroughly just prior to pipetting to insure uniform distribution of cells. This is accomplished by tilting the sample rather than by shaking it. Vigorous mixing should be avoided, since hemolysis may result.

If capillary blood is used, you must work rapidly after the puncture; otherwise, the specimen may clot. You would not necessarily be aware of a partial clot, but this would affect the outcome of a determination. This could result in a low cell count, a low platelet count, or a clogged pipette. Take care not to introduce tissue fluids by forcing blood from the finger.

Hayem's solution should not be more than 3 weeks old, and must be filtered frequently. Occasionally, blood "leaks" from the pipette into the Hayem solution during diluting and causes serious error.

Pseudoagglutination (false clumping) of the red cells occurs in cases of abnormal plasma proteins (myeloma, Hodgkin's disease) or with an increase in cold agglutinins (for example, viral pneumonia). Warming the Hayem's solution to 60° C will counteract the effect of cold agglutinins without hemolyzing the red blood cells. One procedure includes diluting with 0.85 percent (w/v) sodium chloride. Another suggests the use of Gower's solution, which prevents pseudoagglutination.

In polycythemia the number of red cells may be too great to give an accurate count if a dilution of 1:200 is used. In this case, you should draw blood to the 0.3 instead of the 0.5 mark, and then dilute to the 101 mark as usual. The resulting dilution of 1:333 is used in the calculation. In anemia, the opposite condition may be the case. Blood is, therefore, drawn to the 1.0 instead of the 0.5 mark and the sample diluted to the 101 mark. The resulting dilution of 1:100 is used in the calculation, as before.

Sources of error. Sources of error for the RBC manual count arise from improper collection, unclean or moist pipettes, pipettes with broken tips, failure to draw blood to the 0.5 mark, and drawing blood beyond the 0.5 mark. Blood drawn beyond the mark deposits a thin film of cells on the pipette, even though the column of fluid is immediately brought back down to the mark; this practice can produce an elevated cell count.

Diluting fluid should be pipetted in a continuous motion to, but never beyond, the 101 mark. The washing backward and forward of blood and diluent in the pipette to hit the 101 mark exactly overly dilutes the specimen. Slow handling of the specimen after capillary puncture allows some of the blood to coagulate. Mix the blood specimen and diluent thoroughly in the pipette just before charging a hemacytometer. Avoid overcharging or undercharging the counting chamber. Make sure you have an even distribution of cells in the counting chamber, and count all the cells carefully. Do not use diluent that contains debris.

To calculate the cell count, we use the following formula:

$$\text{Average number of RBCs counted in the two chambers} \times \text{dilution (200)} \times \text{depth factor (10)} \times \text{area (5)} = \text{RBCs/mm}^3.$$

The constant factors are depth, dilution, and area. Multiplying the three factors, the result is 10,000. If the standard procedure described above is followed, the number of erythrocytes counted may be multiplied by 10,000 to obtain the total count per mm^3. In other words, we just add four zeros to our count. The range of error for a manual red cell count will usually fall within ± 20 percent, with a minimal error of ± 11 percent and a maximal error of ± 30 percent.

Whenever possible, Gower's solution should be used for manual red counts. Gower's solution includes: Sodium sulfate, 12.5g; glacial acetic acid, 33.3 ml; and distilled water, 200.0 ml. Gower's solution is superior to Hayem's solution in that it prevents rouleaux and clumping of the red cells.

Exercises (012):

1. When shaking the sample after it is introduced into the RBC pipette, why should vigorous mixing be avoided?

2. In cases of pseudoagglutination, what can be done with the Hayem's solution to insure a uniform count?

3. If you draw the blood to the 0.3 mark in an RBC pipette and dilute to the 101 mark, what is the dilution?

4. When might the dilution in exercise Nr. 3 be of practical value?

5. When the blood is drawn beyond the mark in the RBC pipette, what effect will this have on the cell count?

6. Why is Gower's solution considered superior to Hayem's solution?

7. What is maximal range of error for a manual red cell count?

013. Identify normal RBC values and the effects of given factors on the normal values.

Factors that affect red blood cell count. The RBC count may vary in health and disease. For example, a person is reassigned from a seacoast base to one located in a mountainous area. The oxygen content of the air is decreased. The decrease in oxygen stimulates the bone marrow to produce more red cells. Thus, the red cell count rises. Consequently, if a person travels from Miami to Denver, his red cell count may rise from 5.0 to 5.5 million. The red cell count of the person who travels from the mountainous city to a seacoast city will drop.

The normal values for the red blood cell count can be defined within relatively broad limits because wide variations are found in surveys of a large number of supposedly normal persons. Six factors have been known to have statistical significance on the red blood cell count. These factors are:

a. Posture. Counts have been found to be lower if blood is drawn after the subject has been in a recumbent posture.

b. Exercise or excitement. Extreme physical exertion or excitement produces counts significantly higher than those obtained under usual conditions.

c. Dehydration. Severe hemoconcentration can produce red cell counts that are higher than the true value. This condition may be noted in severe burn cases, untreated intestinal obstruction, severe vomiting, or persistent vomiting in pregnancy.

d. Age. The normal RBC count in infants is higher than that of the adult. The count drops rapidly after birth. A gradual rise is noted to the normal adult level and is maintained until old age, at which time there is usually a gradual decrease.

e. Sex. Women usually have a lower RBC count than men; however, no significant difference is noted during puberty and old age.

f. Altitude. As previously described in the example given in the earlier part of this section, residents at high altitudes normally have higher RBC counts than those at sea level.

Normal values. Normal RBC values in millions per mm^3 are as follows:

Birth: 4.8–7.1.
Childhood: 3.8–5.4.
Adult females: 3.6–5.0.
Adult males: 4.2–5.4.

Exercises (013):

Match each normal value or factor that affects normal value in column B with the appropriate population group or effect in column A by placing the letter of the column B item in the space provided in column A. Each column B item may be used once or not at all.

Column A

_____ 1. This factor is primarily responsible for an increase in the RBC count when one travels from Los Angeles to Denver.
_____ 2. This factor will be responsible when the RBC count is noted to be lower after a patient has been in bed for some time as compared to the count taken when he is up and moving around.
_____ 3. After jogging, the RBC is significantly higher.
_____ 4. Due to this factor, an increase in the RBC count may be observed in severe burn cases, severe vomiting, or persistent vomiting during pregnancy.
_____ 5. The RBC count in infants is higher than adults.
_____ 6. Women usually have a lower RBC than men.
_____ 7. Normal RBC values for childhood.
_____ 8. Normal RBC values for the adult male.

Column B

a. Sex.
b. Age.
c. 4.2 to 5.4 million per mm^3.
d. Exercise or excitement.
e. Posture.
f. Altitude.
g. 4.8 to 7.1 million per mm^3.
h. 3.8 to 5.4 million per mm^3.
i. Dehydration.

014. Cite sources of errors for the manual WBC count, give the dilution factors, and calculate the values for abnormal WBC counts.

WBC Counts. You have many occasions in your career to perform white blood cell counts manually. Be sure the counting chamber and pipettes are clean and dry. This is a source of error in the counting of both white and red blood cells. The counting chamber must be scrupulously clean and free of debris which might be mistaken for cells.

In cases where the WBC count is exceptionally high, as in leukemia, the dilution for a white blood cell count should be made in the *red* blood cell diluting pipette. Blood is drawn to the 1.0 mark, and the diluting fluid (2 percent acetic acid) is drawn to the 101 mark. The resulting dilution is 1:100.

In cases of leukopenia, the white pipette should be filled to the 1.0 mark and diluted to the 11 mark with 2 percent acetic acid. The resulting dilution is 1:10. If the number of WBCs in the chamber is still low, all 9 squares (9 mm^2) should be counted. Make the appropriate correction in calculation, dividing by 9 instead of 4.

The calculation for the WBC count is as follows:

$$\frac{\text{Average (of two chambers) number of WBCs counted} \times \text{dilution (20)} \times \text{depth factor (10)} = \text{WBCs/mm}^3.}{\text{area (4)}}$$

Calculation aids in the form of charts and tables may be used. Some of these aids with the accompanying instructions for use are shown in figure 2-1.

If nucleated RBCs are present, the count is corrected by the following formula:

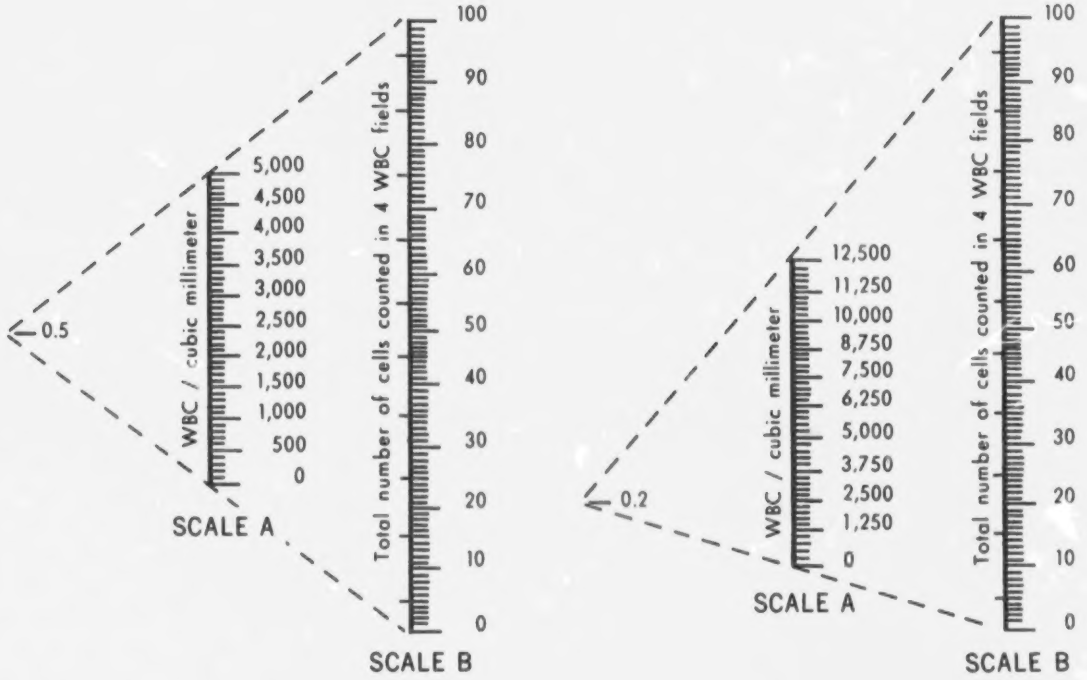
$$\text{Uncorrected white cell count} \times \frac{100}{100 + \text{number of nucleated red blood cells per 100 white blood cells}}$$

The percent of nucleated erythrocytes is obtained from the differential count. The diluting fluid should be free of contamination. Sometimes, small amounts of blood collect in the diluting fluid, which may cause inaccuracies and makes it difficult to distinguish and count the white cells. There is approximately 15 percent error in the manual white blood cell count.

Exercises (014):

1. What are two possible sources of errors in performance of manual WBC count?

2. If there is a case of leukopenia and the dilution factor is used, what would be the total WBC count if 60 cells were counted?



(A) When blood is drawn to the 0.5 mark (1:20 dilution). Instructions: Place a straight edge from the 0.5 point in the left margin across scale A to the number of cells counted in scale B. Read the total count from scale A.

(B) When blood is drawn to the 0.2 pipette reading (1:50 dilution). Instructions: Place straight edge from the 0.2 point in the left margin across scale A to the number of cells counted in scale B. Read the total count from scale B.

I Pipette Reading	II Dilution Factor	III Multiplier	IV Normal Observed WBC Count
1.0	1:10.	25.0	200–400
0.9	1:11.	27.8	180–360
0.8	1:12.5	31.3	160–320
0.7	1:14.3	35.7	140–280
0.6	1:16.7	41.7	120–240
0.5	1:20.	50.0	100–200
0.4	1:25.	62.5	80–160
0.3	1:33.	83.3	60–120
0.2	1:50.	125.0	40–80
0.1	1:100.	250.0	20–40

(C) If blood is drawn in a WBC pipette to the figure in column I, the dilution is as shown in column II. Column III gives the figure to multiply the number of cells counted in one chamber. If the count is normal, the number of cells you would count is given in column IV.

Figure 2-1. Charts for circulating WBC count.

3. In cases where the WBC count is exceptionally high, as in leukemia, what pipette should be used for a WBC count and what is the dilution factor?

4. What is the approximate percent of error in the manual white blood cell count?

5. If you had a white cell count of 15,000 and counted 50 nucleated red cells while counting the 100 white cells of the differential count, what is the corrected white cell count?

015. Point out techniques, reporting, and significance of cerebrospinal fluid (CSF) counts.

Cerebrospinal Fluid (CSF) Counts. CSF is normally water clear and cell free. The specimen is collected in three test tubes, numbered in order of their collection. The third tube or final specimen collected is used for the cell count. If leukocytes are present, they may be counted and differentiated directly in a hemacytometer. Spinal fluid may also be diluted with a fluid which hemolyzes any red cells present and examined on a Wright-stained smear. With the direct method, place an undiluted drop of well-mixed spinal fluid into one counting chamber of a hemacytometer. Examine the entire ruled area for the presence of cellular elements. If both leukocytes and erythrocytes are seen, note the condition of the red cells (fresh or crenated). If the total cell content is low (less than 500), count all the cells and calculate as follows:

$$\frac{\text{Cells counted} \times \text{depth factor (10)} \times \text{dilution (1)}}{\text{area (9)}} = \text{cells/mm}^3$$

If the total cell content is high (over 500) and difficult to count, the specimen can be diluted (1 to 10) in a white cell dilution pipette with Hayem solution and the total cells counted. The calculation then becomes:

$$\frac{\text{Cells counted} \times \text{depth factor (10)} \times \text{dilution (10)}}{\text{area (9)}} = \text{cells/mm}^3$$

The usual technique is to draw the spinal fluid diluent to the 1.0 mark of the WBC dilution pipette; then draw a well-mixed specimen of spinal fluid to the 11 mark of the pipette. Shake the pipette for 2 minutes to mix the specimen. Overshaking may cause the white cells to clump, so this should be avoided. Charge the counting chamber and allow the cells to settle for 5 minutes. Under low power magnification, count all cells in the entire ruled area (9 mm^2). Then switch to high power magnification and examine for the differential count.

$$\frac{\text{Cells counted} \times \text{depth factor (10)} \times \text{dilution (10/9)}}{\text{area (9)}} = \text{cells/mm}^3$$

If more than 100 leukocytes per mm^3 are counted, centrifuge the undiluted specimen, make a smear, and stain with Wright stain. Perform a routine differential count and estimate the ratio of erythrocytes to leukocytes. (NOTE: It may be necessary to use egg albumin or cell-free serum to make the sediment adhere to the slide.) Spinal fluid may be turbid if the cell count is higher than 500 cells per mm^3. If there is gross blood with spontaneous clotting, this indicates a bloody spinal tap. Xanthochromia (yellow coloration) develops after subarachnoid hemorrhage has been present for a few hours and is due to blood pigments. Xanthochromia may also develop from tumors, abscesses, and inflamation.

Cell counts above 10 may be significant. The lymphocyte is the predominant cell in most viral infections, syphilis, and tuberculous meningitis. Pyogenic infections due to meningococci and pneumococci usually result in a predominance of the neutrophil. Cerebral and extradural abscesses, as well as subdural hemorrhages, produce a neutrophilic response although no bacteria can be demonstrated. You should realize that to reflect the true condition of the cerebrospinal fluid, biochemical, bacteriological, viral, serological, and hematological examinations are all necessary.

Exercises (015):

1. What tube is used for the cell count?

2. If erythrocytes are seen with the direct method on the counting chamber, how should they be reported?

3. In performing a manual routine cell count on CSF, you count 9 cells in the undiluted specimen. What is the reported cell count?

4. In the usual technique when spinal fluid diluent is used, why should overshaking be avoided?

5. If more than 100 leukocytes are counted, what must be done with the undiluted specimen?

6. Of what general value is a CSF differential to the physician?

016. Indicate the procedures for semen analysis in terms of instructions to patient, procedures, reagent, calculation of sperm counts, normal count, and stains.

Semen Analysis. Semen analysis also involves a cell count. However, several other aspects should be considered first in this procedure. Semen analyses may be requested by a physician in the diagnosis of infertility or as a followup test after vasectomy.

Instructions to patient. Instructions to the patient for semen collection must be explicit and explained in a professional manner. Usually the attending physician will give these instructions; however, the technician should remind the patient of several critical points. First, the patient may be required to abstain from intercourse for a period directed by the physician. The best container for collection is a clean, widemouthed, glass jar; but if a condom must be used, it should be thoroughly washed, rinsed, and dried before using. All of the specimen must be collected, since the sperm count varies considerably in different portions. You should also explain that the specimen must be delivered to the laboratory as soon after collection as possible, preferably within 30 minutes and never over 2 hours. During this time the specimen should be kept at room temperature (25° C) and not subjected to extremes of heat and cold.

Gross examination. Upon receipt of the specimen, you should record the time received and the time of collection. Then measure the volume in a 10 ml graduated cylinder. At this time, observe and record the color (white, grey, yellow, etc.), turbidity (clear, opalescent, opaque, etc.), and viscosity (viscid, gelatinous, liquid). Finally, in this gross examination, determine the pH with a pH reagent strip and record this.

Examination for motility. Within 15 to 30 minutes after collection, the semen will liquefy from the action of fibrinolysin. When the specimen has become fluid, place 1 drop on a slide and place a coverslip on it. Do not delay in performing the motility examination. Under high dry power, count motile and nonmotile spermatozoa in two or more areas. Only those which move forward actively are considered motile. Record the percent of motile forms seen. Repeat this procedure in 3 hours and again in 6 hours, using a new drop from the original specimen each time. Do *not* incubate at 37° C, since this impairs motility.

Sperm count. The sperm count is made when the specimen has become fluid. Mix the specimen thoroughly and dilute it 1:20 with a fixative solution containing 5 gm sodium bicarbonate, 1 ml formalin, and 100 ml of distilled water. This dilution may be made by pipetting 0.1 ml of semen and 1.9 ml of the diluting fluid. Let the mixture stand until the mucus dissolves. Shake it thoroughly and load a hemacytometer. Count the spermatozoa in the same manner as you would count RBCs. The calculations are as follows:

$$\text{Sperm count} \times 20\ \text{(dilution)} \times 5\ \text{(area)} \times 10\ \text{(depth factor)} = \text{sperm/mm}^3$$

Then convert your answer to sperm/ml by multiplying the above answer by 1,000.

Example:

$$155 \times 20 \times 5 \times 10 = 155{,}000\ \text{sperm/mm}^3$$
$$155{,}000 \times 1{,}000 = 155{,}000{,}000\ \text{sperm/ml}$$

Normally, a cubic millimeter of semen contains 60–150 million spermatozoa.

Morphological examination. To study the morphology of spermatozoa, make a smear of semen and allow it to air dry. Then flush with diluting fluid to dissolve mucus and wash with Wright buffer. Stain the smear with Wright or Giemsa stain. Examine it with the oil immersion lens and record the percent of abnormal forms. The morphology examination may also be observed while doing the count. Morphologically normal sperm are quite uniform in appearance. Any sperm with rounded, enlarged, small, or bilobed heads are abnormal. Abnormal tails may also be seen as enlarged, small, irregular in length, absent, or multiple.

Morphological examinations of semen smears are included in the context of hematology because this test is frequently performed in the hematology department of many laboratories. Both the sperm count and the stained smear are performed with materials common to the hematology sections of clinical laboratories.

Exercises (016):

1. Why must all of the semen be collected by the patient?

2. How soon after collection should the specimen be delivered to the laboratory?

3. What two things must be recorded upon receipt of the specimen?

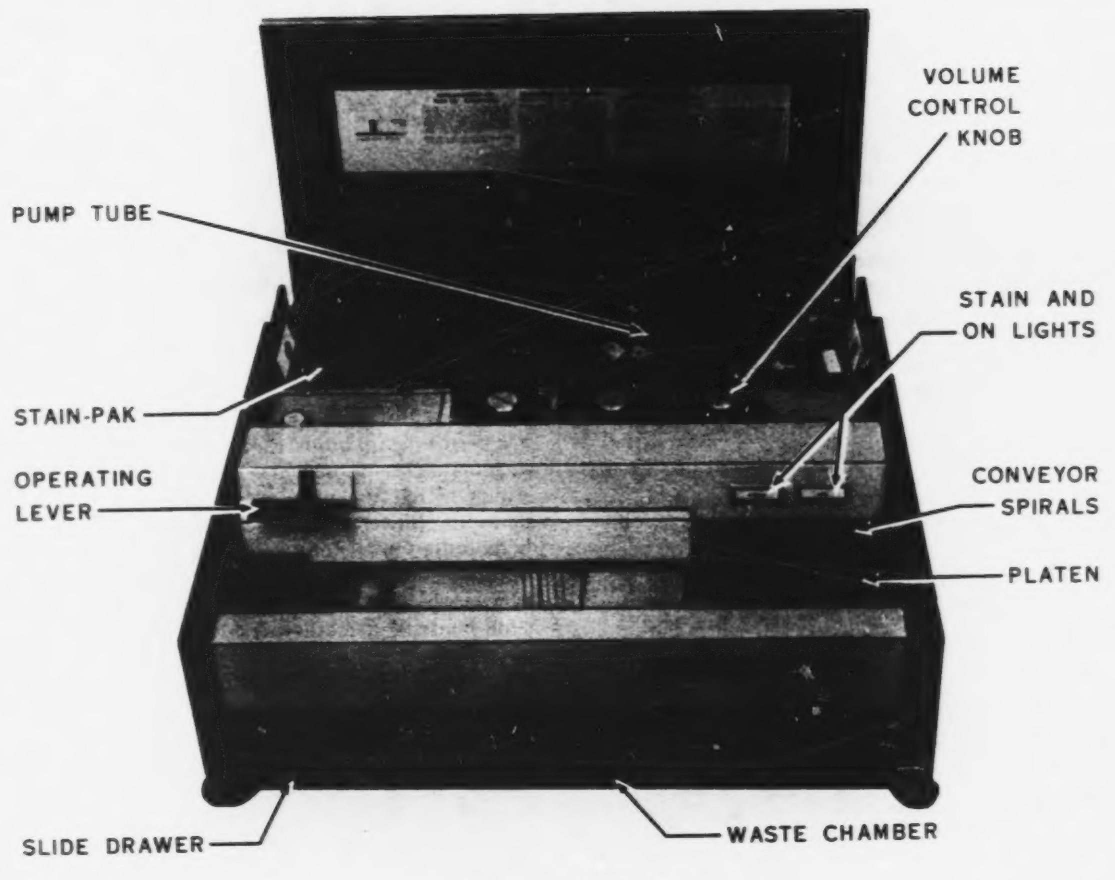


Figure 2-9. Hema-Tek Slide Stainer.

stained at a rate of 1 per minute, and after staining and rinsing, they are blown dry by a low velocity blower and then deposited in the slide drawer at the end of the machine. Figure 2-9 illustrates the essential components of this machine.

There are two *conveyor spirals* which are turned by a conveyor drive motor. These spirals hold the glass slides in place, moving them through the staining process. If slides are not properly aligned between both conveyor spirals, they will break.

The *platen* is a platform which separates the two conveyor spirals. It supports the slides as they are carried through the staining process.

There are three *volume control knobs* which serve to control the amount of solution delivered to each slide. For efficient operation, the platen should be cleaned periodically throughout the day with methanol, using soft clean gauze.

Staining Times. Generally, when bone marrow smears are stained, the staining times are increased. The staining times for both bone marrow and peripheral smears may vary from one laboratory to another. This is due to the Wright's stain and the pH of the buffer. Occasionally, a new lot of Wright's stain is used and will then require a change in the staining times.

Exercises (025):

1. What is the term for stains used to stain living cells?

2. What are the constituents of Wright's stain?

3. What purpose does the alcohol serve in the stain?

4. When using the staining rack method for staining blood smears, how should the stain be applied?

5. If the stain-buffer mixture is not evenly distributed, what is recommended?

6. At what stage of the staining does most of the staining occur?

4. Within 15 to 30 minutes after collection, what will cause liquefaction?

5. For semen specimen collected at the laboratory, at what time intervals following collection should you examine it for motility?

6. Why is incubation of specimen at 37° C not recommended?

7. What reagents are used for diluting semen?

8. What effect does the diluting fluid have on the spermatozoa?

9. You mix 0.1 ml of semen with 1.9 ml of diluting fluid, and you perform the count as you would a routine RBC count. If you count 127 cells, what is the reported count in number per ml?

10. What is the normal sperm count?

11. What two stains are recommended for smears of semen?

017. Identify the principles of the given automated methods for counting blood cells and the functions related to these principles.

Automated Methods. There are at least two automated methods available for counting blood cells. One of these methods is an *optical system* based upon the production of light impulses. In this system, the cells are diluted and drawn through the counting zone by a positive displacement metering pump. As cells pass through the counting area, they produce photoelectronic impulses which can be counted. Another method of automating the counting of blood cells utilizes the *principle of resistance* in an electrical field. Since blood cells are poor electrical conductors, they act as an impedance to current flow. As more cells pass into the electrical field, they offer correspondingly more resistance. The change in the current flow caused by the change in resistance is sensed and counted by a digital counting apparatus. The digital counter is designed to produce numbers in a range which approximate the number of blood cells that cause the resistance.

Devices with optical systems. Several instruments employ a photoelectronic counting device which uses an optical sensing system and an electronic counting system. This is used in the Fisher Autocytometer, Technicon Autoanalyzer, and the SMA-4, SMA-7, and Hemalog electronic instruments. As the diluted blood specimen enters the counting area, the sample is "inspected" by a reversed dark field microscope arrangement. A schematic presentation of this arrangement appears in figure 2-2. When no particles are present in the sample, the narrow light beam from the lamp passes straight through to a dark field disc, which blocks further passage. However, any suspended particles, such as blood cells, will interrupt the light beam. Scattering of the light beam due to the mass of each blood cell causes a light flash to pass around the dark field disc. The light flash is focused into a detection system and converted into an electrical pulse that can be counted.

Impedance (resistance) counters. If a suspension of erythrocytes in an electrolyte is drawn through an aperture having electrodes on each side to form an electrical circuit, the system can be utilized to count the red blood cells in suspension. As the blood cells pass through the aperture, the mass of the cell changes the resistance between the electrodes. This is used in the Celloscope, Coulter, and General Science Corporation Hematology Count systems. This changing of the resistance alters the current flow and causes electronic pulses as a result of the variation in the field. The changes are amplified, inspected, and counted electrically. The end product is a number which represents the number of blood cells in the sample being counted. By arranging the pulses, the sampling volume, and sampling time, this device can be used to report directly the number of cells per cubic millimeter. Figure 2-3 is a schematic of the operation of one commercial instrument that counts blood cells by

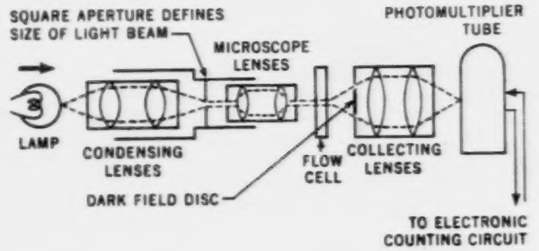


Figure 2-2. Schematic of photoelectric particle counting system.

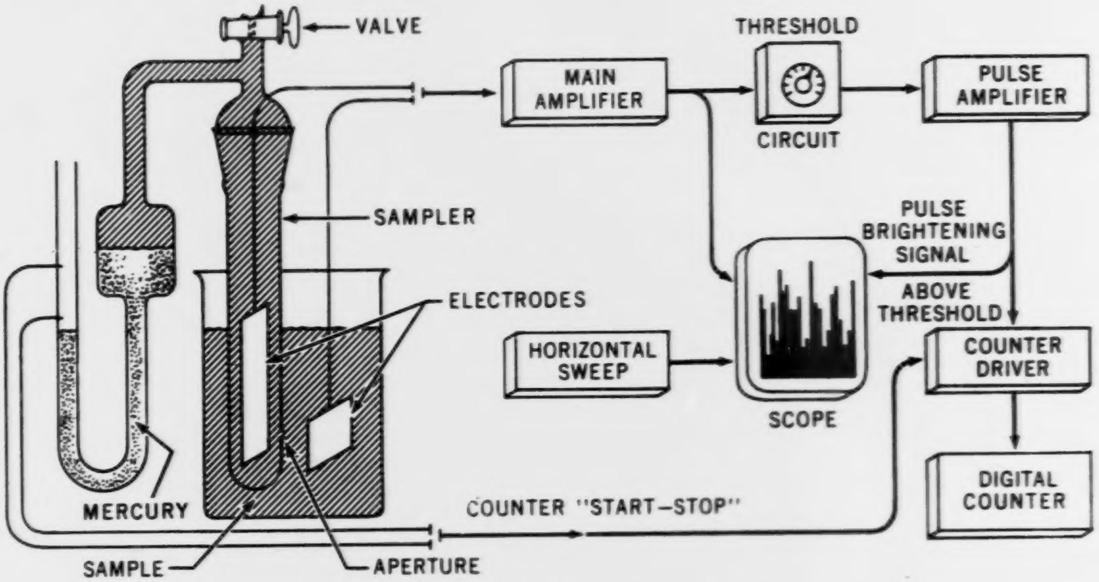


Figure 2-3. Schematic of a resistance-impedance of electronic counting apparatus.

using the electronic principle of resistance change. When the valve is opened, the mercury falls, thus creating a vacuum in the sampler. This change in pressure causes cells in the sampler to be sucked through the aperture and into the sampler. The removal of cells from the sample causes a change in electrical conductivity between the two electrodes. This change is amplified and displayed on the scope. It is further amplified and registered on the digital counter. The higher the count, the greater the probability that more than one cell will enter the aperture at one time (coincidence passage). For this reason, white blood counts over 10,000 and all red blood counts are corrected for coincidence passage.

Exercises (017):
Match each of the column B items with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

____ 1. Based upon the production of light impulses.

____ 2. Based on the principle that blood cells are poor conductors of electricity and each passing cell momentarily decreases a flow of current between two electrodes.

____ 3. As the diluted blood specimen enters the counting area, the sample is inspected by a reversed dark field microscope.

____ 4. Scattering of the light beam due to the mass of each blood cell causes a light flash to pass around the dark field disc.

____ 5. As the blood cells pass through the aperture, the mass of the cell changes the resistance between electrodes.

____ 6. When the valve is opened, the mercury falls, thus creating a vacuum in the sampler.

____ 7. The higher the count, the greater probability that more than one cell will enter the aperture at one time.

Column B

a. Principle of resistance.
b. Impedance (resistance) counters.
c. Devices with optical systems.
d. Optical system.
e. Coincidence passage.

018. Match a list of the components of the model FN Coulter Counter with their functions.

Automated Counting Equipment. Automated and electronic equipment used for enumerating the formed elements in the blood have several advantages over the hemacytometer techniques. Mainly these types of equipment provide methods which have greater precision (reproducibility), as indicated by a lower coefficient of variation and the capacity for completing a large quantity of determinations quickly and without increasing error due to fatigue. The cost of the electronic cell-counting instruments runs into several thousands of dollars. Nevertheless, if a large number of determinations are to be made each day, the economic factors will justify their use in the laboratory. Some of these instruments common to Air Force laboratories are discussed briefly in the following section.

Coulter Counter Model FN. The Coulter model FN is one of the most widely used pieces of equipment in the hematology laboratory for counting of red and white cells. As we briefly discuss some essential components of this model, refer to figure 2-4.

Readout assembly. This unit displays the total in easy-to-read bright red nixie tube numerals. When the instrument has stopped counting, the numbers are read directly from the unit.

Dual monitor system. This system consists of an oscilloscope and a mirror projection screen.

(1) The oscilloscope screen displays an electronic pattern in the form of spikes or pulses for each particle counted. In instances of interfering debris, an abnormal, or irregular, pattern is shown.

(2) The microprojection screen displays a constant view of the small hole in the aperture tube through which the sample passes. The user is able to detect any debris which may block the opening during a count.

Sensitivity controls. These consist of the attenuation control, the aperture current control, and the threshold dial. When the instrument is set up for the first time, calibration must be made with these settings. Thereafter, as long as the size particles that are being counted remain the same, recalibration is not necessary. Periodic check is recommended in accordance with the manufacturer's instructions.

(1) The attenuation control determines the overall sensitivity of the electronic amplification.

(2) The aperture current control determines the amount of current which passes between the two electrodes in the sample stand assembly.

(3) The threshold dial determines the level above which the pulses of the particles will be counted.

Power switch. This turns the instrument on and off.

Sample stand assembly. This assembly consists of the beaker platform, the aperture tube, two platinum electrodes, the control piece, the mercury manometer, vacuum pump controls, and aperture positioning controls.

(1) The *beaker platform* holds the sample beaker and must be positioned so that the aperture tube is very close to the bottom of the beaker but does not touch it.

(2) The *aperture tube* has a very small orifice or hole through which the diluted sample passes. For red and white cell counts, the aperture tube with an orifice of 100 μ size is recommended.

(3) The *two platinum electrodes* are on the assembly stand. The external electrode is located outside the aperture tube. During a count, this electrode *must* be immersed in the diluted sample. The internal electrode is found inside the aperture tube.

(4) The *control piece* connects the aperture tube to the mercury manometer and has two stopcocks. When closed, both stopcocks will be in the horizontal position. When both are turned until they lie in a vertical position, they are open. When counting, the vacuum control stopcock is used. Negative pressure is introduced, thus causing the mercury in the manometer to fall. To fill and flush the instrument, the stopcock located below and to the left of the vacuum control stopcock is used. At the top of the control piece is located a rubber tubing connection that leads to a "T" tube, which, in turn, is connected to the vacuum regulator and to the waste flask. There is another opening below this unit and behind the flushing stopcock which is connected to the electrolyte solution by another rubber tube.

(5) The *mercury manometer* (fig. 2-5) is used to control the exact amount of diluted sample to be counted. For red and white cell counts 0.5 ml of diluted sample is counted. Three electrodes are systematically located in the manometer. One is responsible for starting the particle count, and another stops the particle count as soon as 0.5 ml of diluted sample has been drawn through the orifice of the aperture tube. The third electrode serves as a ground.

(6) The *vacuum pump* controls the mercury level in the manometer and is controlled by the vacuum control regulator located above the pump. A piece of tubing attached to the vacuum pump is pinched off at a set length. This is the vacuum limit adjustment; thus the length should not be changed once it has been set.

(7) The *aperture positioning controls* located at the bottom, near the front of the instrument, are used to make slight corrections in the position of the aperture tube so that it can be seen on the microprojection screen.

Exercises (018):

Match each of the essential components of the Model FN Coulter Counter in column B with statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

_____ 1. When the instrument has stopped counting, the numbers are read directly from this unit.

_____ 2. Consists of an oscilloscope and a microprojection screen.

_____ 3. Displays an electronic pattern in the form of spikes or pulses for each particle counted.

_____ 4. Through this, the user is able to detect any debris

Column B

a. Microprojection screen.
b. Attenuation control.
c. Threshold dial.
d. Oscilloscope screen.
e. Dual monitor system.
f. Readout assembly.
g. Aperture current control.
h. Mercury manometer.
i. Vacuum pump.
j. Control piece.
k. Vacuum control stopcock.

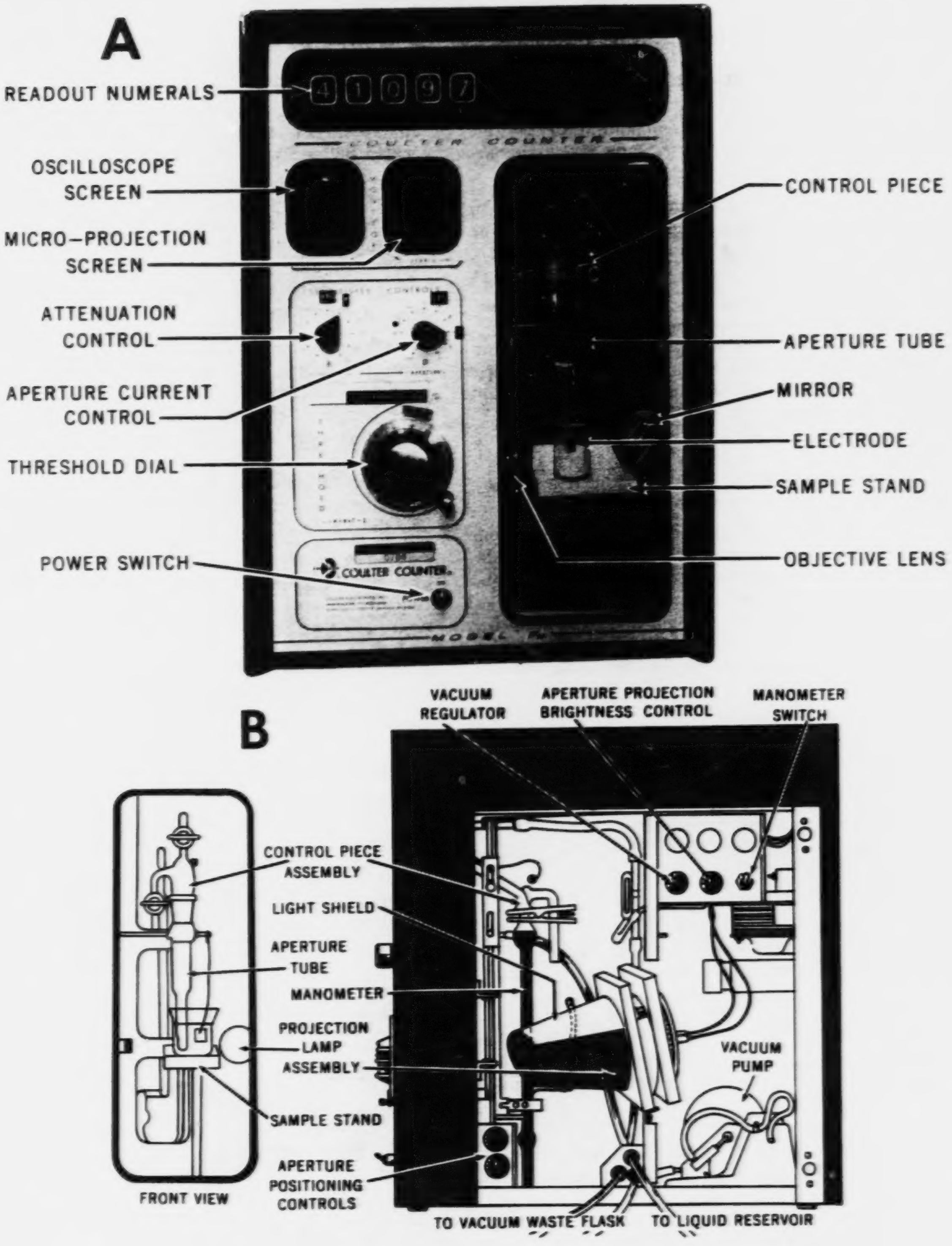

A
READOUT NUMERALS
41097
OSCILLOSCOPE SCREEN
MICRO-PROJECTION SCREEN
ATTENUATION CONTROL
APERTURE CURRENT CONTROL
THRESHOLD DIAL
POWER SWITCH
CONTROL PIECE
APERTURE TUBE
MIRROR
ELECTRODE
SAMPLE STAND
OBJECTIVE LENS
COULTER COUNTER
B
VACUUM REGULATOR
APERTURE PROJECTION BRIGHTNESS CONTROL
MANOMETER SWITCH
CONTROL PIECE ASSEMBLY
LIGHT SHIELD
APERTURE TUBE
MANOMETER
PROJECTION LAMP ASSEMBLY
SAMPLE STAND
APERTURE POSITIONING CONTROLS
FRONT VIEW
VACUUM PUMP
TO VACUUM WASTE FLASK
TO LIQUID RESERVOIR

Figure 2-4. Coulter Counter Model FN. A - Front view; B - Side (right).

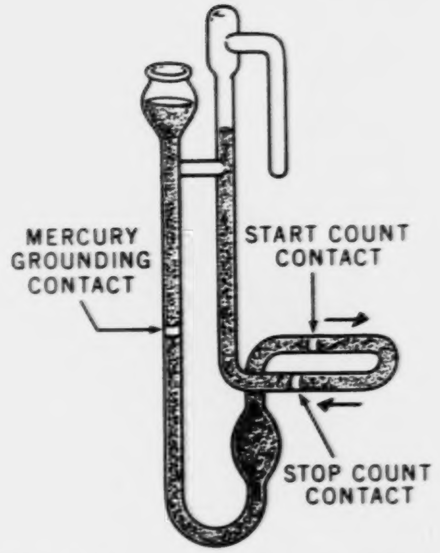


Figure 2-5. Manometer, Coulter Counter, Model FN.

which may block the orifice during a count.

____ 5. Displays a constant view of the small hole in the aperture tube through which the sample passes.

____ 6. Determines the overall sensitivity of the electronic amplification.

____ 7. Determines the amount of current which passes between the two electrodes.

____ 8. Determines the level above which the pulses of the particles will be counted.

____ 9. Consists of the beaker platform, the aperture tube, two platinum electrodes, the control piece, and the mercury manometer.

____ 10. Has a very small orifice or hole through which the diluted sample passes for red and white counts.

____ 11. This electrode is located outside the aperture tube and must be immersed in the diluted sample.

____ 12. Connects the aperture tube with the mercury manometer.

____ 13. Are open when they both lie in a vertical position.

____ 14. This control stopcock is used when counting; negative pressure is introduced, causing the mercury in the manometer to fall.

____ 15. This stopcock is used to fill and flush the instrument.

____ 16. Used to control the exact amount of diluted sample to be counted.

l. Left control stopcock.
m. Aperture tube: orifice 100 μ.
n. Aperture tube: orifice 50 μ.
o. Sample stand assembly.
p. External platinum electrode.
q. Internal platinum electrode.
r. Aperture positioning controls.

____ 17. Contains three electrodes; one starts the count, another stops the count, and the third acts as ground.

____ 18. Controls the mercury level in the manometer.

____ 19. Used to make slight corrections in the position of the aperture tube so that it can be seen on the microprojection screen.

019. Point out sources of error, problems, and precautions in the operation of the Coulter Counter for red and white cell counts.

Problems of Electronic Counting—Coulter Counter. Although not a part of the Coulter Counter operation, errors of cell counting due to dilution inaccuracies are considered among the most common. Automatic diluters can contribute an additional slight error over careful manual pipetting. However, automation does reduce the personal dilution variation among technicians. Another common problem with the electronic counting instruments is the indication of erroneous impulses. The instruments are particle counters and cannot differentiate blood cells from other solids. False impulses result from electronic problems such as inadequate electrical ground or a lack of shielding from external electronic noise. Centrifuges with worn arcing brushes are excellent electronic noise generators. A background count using a clean diluent with a known low count should identify these sources of error. Contaminating particles in apparently clear diluent are another source of impulses. A sealed bottle of saline for injection is no guarantee that the saline is suitable as a diluent. Background counts must be made on all diluents as a part of the daily quality control program.

When using 0.85 percent sodium chloride as a diluent, Zaponin® or saponin should be used as a lysing agent. When Isoton® is used as the diluent, Zap-Isoton® should be used as the lysing agent. When the diluted blood sample turns crystal clear, it is ready to be counted. The time interval for completion of hemolysis will vary, depending upon the type of saponin used. The diluted sample should not stand more than 10 minutes; otherwise destruction of the white cells by saponin will begin to occur. If Zap-Isoton® is used, the red cells will be lysed immediately, but the white cells will be preserved up to 30 minutes. Before each set of red or white counts is performed, a background count should be performed. This consists of doing a count on the diluent used to insure that falsely elevated counts are not being obtained due to contaminated diluent or sample beakers. Background counts of 100 per mm^3 or less are acceptable and no correction is required on the cell counts.

Precautions. Certain precautions should be observed when using electronic particle counters. First of all, keep in mind that running one control does not control an entire group of procedures. Errors may still be introduced with individual samples. Most laboratories allow a ±2 percent variation for different dilutions of the same sample. Bubbles must be avoided since they will be counted as cells. When all is going well, the oscilloscope pattern will show steady peaks of approximately the same height. Flashes on the screen usually indicate a plugged orifice or contaminating debris. It is well to flush the orifice between counts with diluent. Replace the plastic tubing periodically to avoid cumulative contamination. Calibrating instructions for commercial instruments are available from the manufacturer. If you encounter problems such as continuous counting, failure to count, and loss of vacuum, consult the medical equipment repair personnel.

Maintenance. One of the most significant types of maintenance is keeping the instrument clean. Rinsing periodically during the day with Isoterge® proves quite valuable in this respect.

To reduce incidence of instrument failures, do the following on a daily basis:

a. Observe the mercury travel time in the manometer. If the mercury column does not move, moves erratically, or flows quickly into the aperture tube, the manometer needs cleaning. The counting cycle should be between 12 and 15 seconds for accurate results. A cycle less than 12 seconds may suggest a broken orifice. Cycles greater than 15 seconds usually indicate a dirty orifice or dirty manometer and mercury.

b. When Isoton® and Zap-Isoton® are used, one threshold setting can be used for both white and red blood counts.

c. During the counting procedure, keep all objects, including hands, away from the machine until the count is completed.

d. Always make sure that the external electrode is submerged in the diluted sample.

e. The liquid level in the waste flask should be kept below the glass tubing at all times. If the level is too high, it may be drawn into the vacuum pump, which may then become damaged.

f. There should never be air bubbles or splits in the mercury in the manometer.

g. When the instrument is not in use, it should be left filled with either Isoterge® or Isoton®.

In addition to the daily maintenance, other maintenance should be performed periodically. Once each week do the following:

- Oil the vacuum pump.
- Clean the aperture tube; be very careful with the orifice insert.
- Check threshold zero.

Once every 6 months you should:

- Change the latex tubing.
- Change mercury.

Exercises (019):

1. List the most common sources of error in electronic cell counting.
2. How long should the diluted sample be allowed to stand after the addition of the saponin agent before the destruction of leukocytes begins to occur?
3. If Zap-Iostom® is used, how long will the leukocytes remain preserved before destruction occurs?
4. What is the acceptable background count prior to performing each set of red or white cell counts?
5. Why must bubbles be avoided in the sample vial?
6. What do flashes on the screen usually indicate?
7. For accurate results, what is the counting time of the Coulter counter?
8. What does a cycle of greater than 15 seconds indicate?
9. Why should the liquid level in the waste flask be kept below the glass tubing?
10. How often should the aperture tube be cleaned?

020. Match types of cell counts performed by the Coulter Counter® Model S with the principles used in counting these values, and match component parts of the Model S Coulter with their functions.

Coulter Counter® Model S. The Coulter Counter Model S has significantly enabled the hematology laboratory to cope with the ever-increasing workloads. Blood samples may be introduced at a rate of one every 20 seconds. Results are received on a printed card, and the analysis is completed 40 seconds after the sample has been introduced into the instrument. The analyzer and diluter units of the Coulter Model S are shown in figure 2-6.

Principle. The Model S Coulter uses the impedance principle for WBC, RBC, and MCV (mean cell volume) counts. The hemoglobin is converted to cyanmethemoglobin and measured by a photocell. The PCV (packed cell volume), MCH (mean cell hemoglobin), and MCHC (mean cell hemoglobin concentration) counts are calculated by the analyzer.

Components. The instrument consists of five connected units:

a. Diluter. The diluter aspirates, pipettes, dilutes, mixes the blood, lyses the red cells, and "senses" (for example the changes of current between electrodes) the hemoglobin at the photodevice.

b. Analyzer. The counting, measuring, and computing of the results take place in this unit.

c. Power supply. The voltage required to run the electronic system is supplied through this unit.

d. The printer. This unit receives the digital information from the power supply and prints these results on report cards.

e. The pneumatic power supply. This unit furnishes the diluter with the necessary vacuum and pressure.

The analyzer consists of (1) the electronic cards and (2) the oscilloscope module and system monitors. In this analyzer, the voltage pulses are counted for 4 seconds, amplified, and shown on the oscilloscope screen. Three red counts are simultaneously counted, and the average is made and recorded. When debris, dirt, or malfunction is present in any of the apertures, the corresponding data rejection light will come on, and that red or white count will not be used. When two of the counts are rejected, the three data rejection lights will come on and a count of 0.0 will be recorded. Further information is outlined in the manufacturer's operating manual for instructional guidelines and additional functions of components.

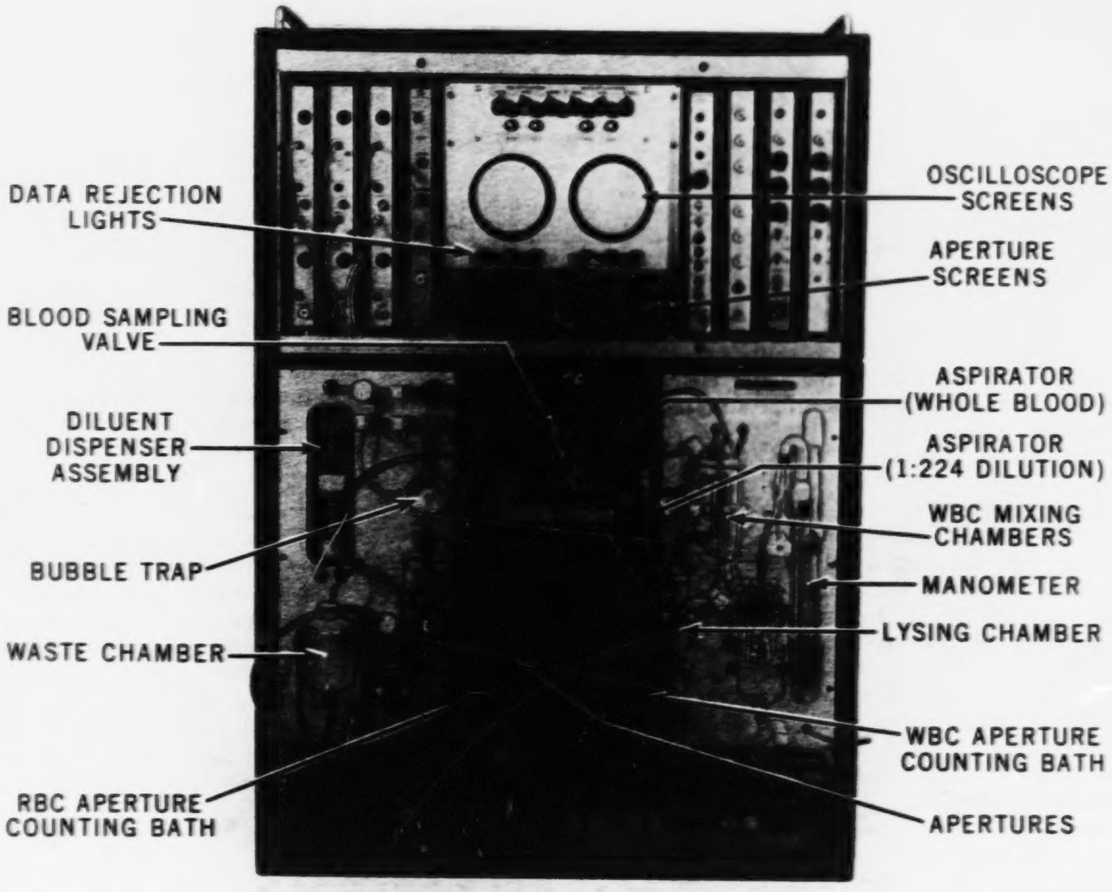

DATA REJECTION LIGHTS
BLOOD SAMPLING VALVE
DILUENT DISPENSER ASSEMBLY
BUBBLE TRAP
WASTE CHAMBER
RBC APERTURE COUNTING BATH
OSCILLOSCOPE SCREENS
APERTURE SCREENS
ASPIRATOR (WHOLE BLOOD)
ASPIRATOR (1:224 DILUTION)
WBC MIXING CHAMBERS
MANOMETER
LYSING CHAMBER
WBC APERTURE COUNTING BATH
APERTURES

Figure 2-6. Diluter and Analyzer units, Coulter Counter, Model S.

Exercises (020):

Match each column B item with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or not at all.

Column A

____ 1. The Model S Coulter uses the impedance principle in counting these values.

____ 2. Compound is converted and measured by a photocell.

____ 3. Are calculated by the analyzer.

____ 4. Aspirates, pipettes, mixes, dilutes, lyses the red cells, and "senses" (for example, the changes of current between electrodes) the hemoglobin at the photodevice.

____ 5. Counts, measures, and computes results.

____ 6. Provides the necessary voltages required to run the electronic system.

____ 7. Receives the digital information from the power supply and prints these results on report cards.

____ 8. Furnishes the diluter with the needed vacuum and pressure.

____ 9. Consists of the electronic cards, oscilloscope module, and system monitors.

____ 10. Voltage pulses are amplified and counted.

Column B

a. WBC, RBC, MCHC.
b. WBC, RBC, PCV.
c. WBC, RBC, MCV.
d. HCB.
e. PCV, MCH, MCHC.
f. Power supply.
g. Printer.
h. Pneumatic power supply.
i. Analyzer.
j. Diluter.
k. Oscilloscope screens.

021. State the principle of the Laser Hematology Counter in terms of its operation, operating hints, and limitations.

Laser Hematology Counter. The Laser Hematology Counter is an automated system for the quantification of blood cells. The instrument is considered as four subsystems which are mechanical, optical, electronic, and data presentation. The instrument dilutes, lyses, and measures red and white blood cells at the rate of 60 samples per hour. In addition, it provides hemoglobin determination by the cyanmethemoglobin method. It measures the hematocrit and calculates MCV, MCH, and MCHC. Note the illustration of Laser Hematology Counter, the Hemac 630L, by Ortho, in figure 2-7.

Principle. Particles when exposed to a source of illumination scatter and absorb light when the illumination is an intense, direct, straight line source. The extinction resulting from the light which is scattered can be measured. The measure can be used to count and size particles.

Operation. In the operation, the cells are transported through the optical system of the instrument in liquid suspension. The sample is injected into a stream of sheath salac (saline diluent, 11 ml per test), and laminar flow conditions are maintained so that the salac stream carries the sample through the optical system without mixing. The stream is narrowed in a glass to 250μ, but the sample stream of cells contained in its center is confined within 18μ. At this point, a beam of light from the laser is focused to intersect the central cell stream, as shown in figure 2-8. The light, which appears at uniform intensity, is focused along a fixed plane, through which the individual cells are made to flow. Some of the light is scattered as it passes through the cell. The loss in light that does not continue along the fixed plane or extinction is measured by the sensor, as indicated in figure 2-8.

The sensor, in response to the light signals, generates electrical pulses having magnitudes relative to the measured optical property. Simultaneously, the sensor signal is fed into a series of electronic counters and register. Here the measured information is processed and stored until it is ready to be printed at the end of the operating cycle.

Limitations. Limited ranges of the instrument have been determined by the manufacturer and given below:

Red blood cells	500,000–8,000,000 cells/mm^3
White blood cells	0–75,000 cells/mm^3
Hemoglobin	3–23 g/dl
Hematocrit	9–66 percent

Samples falling above these limits must be diluted prior to their introduction into the instrument. Samples falling below these limits cannot be accurately measured. Additional information may be obtained from the operator's manual published by the manufacturer.

In order to complete the cyanmethemoglobin reaction in a relatively short time, the operational parameters, such as pH, reagent concentrations, lysing agent, and blood/reagent ratio, have been altered in the Hemac system. Despite the alteration, the Hemac system produces a chromogen whose absorbance spectrum is like that of cyanmethemoglobin.

Operating hints. The following operating hints are helpful for the efficient operation of the instrument:

(1) Observe cleanliness of flow channel routinely for freedom from dust, debris, fingerprints, and air bubbles.

(2) Always invert/mix sample before withdrawing a portion for analysis.

(3) Never sample with less than 0.2 ml in the container.

(4) Wait for READY light before introducing a new sample.

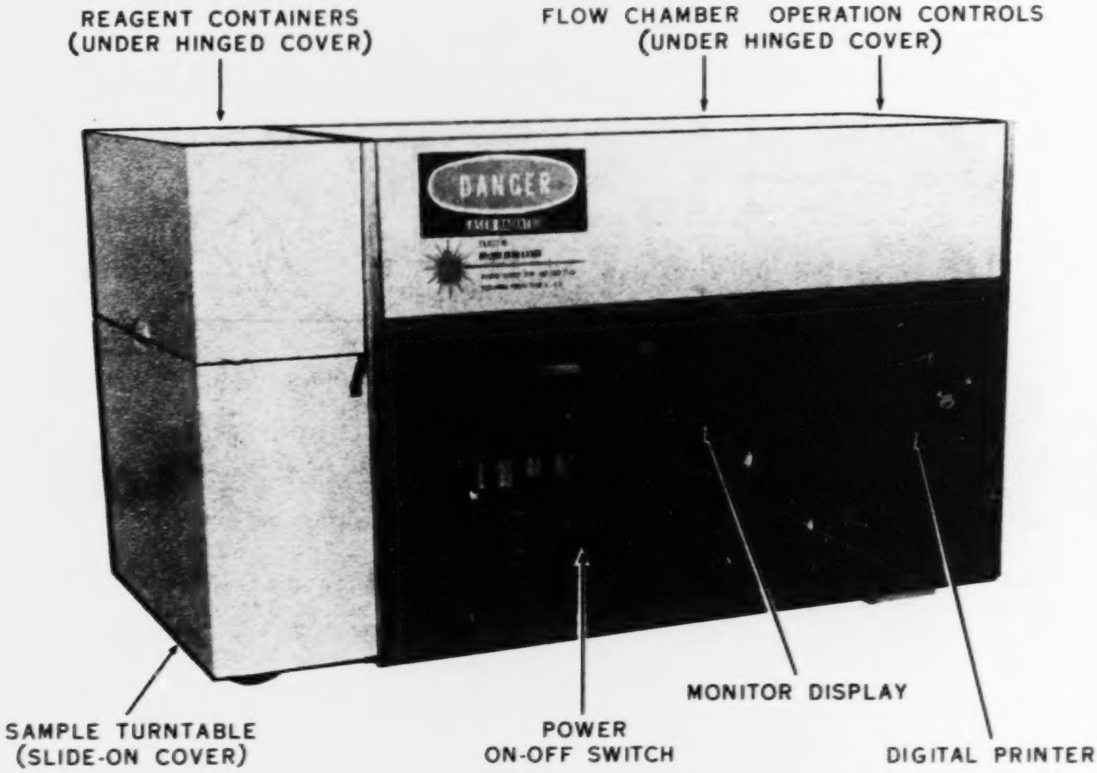

REAGENT CONTAINERS
(UNDER HINGED COVER)
FLOW CHAMBER
OPERATION CONTROLS
(UNDER HINGED COVER)
DANGER
SAMPLE TURNTABLE
(SLIDE-ON COVER)
POWER
ON-OFF SWITCH
MONITOR DISPLAY
DIGITAL PRINTER

Figure 2-7. Hemac 630L Laser Hematology Counter.

(5) Observe sensor signal on the monitor scope during RBC and WBC counting. Check for proper amplitude and pattern.

(6) Leave the cleaning solution sample in the loading tray as an indication of the instrument status.

(7) Replace near-empty reagent containers as soon as possible to avoid getting air into the system.

(8) Avoid shaking the reagent fill tube while changing containers, since this will permit air to enter the system.

(9) Routinely inspect slide valves, pumps, poppet valves, and lines for signs of leaking.

(10) Check reagent containers every 200 samples.

Exercises (021):

1. What are the four subsystems of the laser hematology counter?
2. By what method does the instrument determine hemoglobin?
3. How are the hematocrit and indices determined?
4. Based on the principle related to the laser hematology counter, what happens to particles when exposed to a source of illumination?
5. What happens to the extinction resulting from the scattered light? And how is it used?
6. In the operation of the instrument, primarily through what system are the cells transported?
7. What light is measured by the sensor?

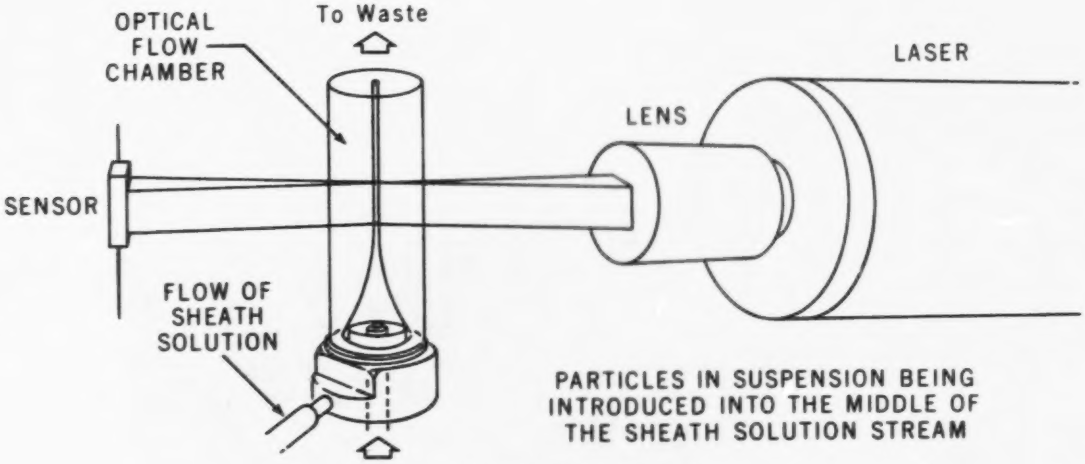

OPTICAL FLOW CHAMBER
To Waste
LASER
LENS
SENSOR
FLOW OF SHEATH SOLUTION
PARTICLES IN SUSPENSION BEING INTRODUCED INTO THE MIDDLE OF THE SHEATH SOLUTION STREAM

Figure 2-8. Cell sizing and counting.

8. In response to the light signal, what action does the sensor produce?

9. Where is the sensor signal sent?

10. In using the laser hematology counter, when the hemoglobin value is 27 g/dl, what should be done with the sample?

022. Point out some sources of error in blood counts that are most common to electronic counters.

Sources of Error. Much confidence is placed in the accuracy and reliable performance of sophisticated electronic devices. Consequently, there is an inclination to accept the results of electronic cell counters as being accurate and beyond question. These assumptions are false, since each performance varies with the component quality and design. Moreover, there are considerations other than the electronic system which can have significant bearing on the results. Below is a brief list of sources of error:

Labeling. Incorrect labeling of patient source or sample number may occur. This should be a prime consideration when the machine results do not correspond with the clinical situation.

Dilution. In the Coulter counting system, an error in diluting will not only create an error in the calcumulated red cell count, but it will yield an error in the hematocrit, which is automatically computed for models B, D, F, and S. It may also produce an error in the MCV if it is calculated by the technician from the red cell count. If, however, the MCV is automatically computed by Model S or the accessory for Models B, D, or F, it is not affected by erroneous dilution.

Presence of cell types not to be counted and destruction of cells to be counted. Red cells must be lysed to avoid interfering counts. Erroneous high counts may be produced if there are residual erythrocytes or stroma particles due to incomplete lysis. Incidents have occurred in which blood from chronic lymphocytic leukemia patients gave falsely low leukocyte counts due to the cytolytic action of the lysing reagent used. Fragile leukemia cells may disintegrate, and the total white count should be activated from the slide as a check on the Coulter counter result.

Other sources of error. Other potential sources of error include:

a. Error in metered delivery of cells after dilution due to pump, valves, tubing, connections, or cutoff switch.

b. Partial obstruction of aperture tube in impedance type instrument.

c. Coincidence loss. This occurs when two cells enter the sensing zone of the apparatus simultaneously or when the second enters immediately after the first but in the brief refractory period as the signal from the first is developed.

d. False signals from electrical or radio frequency (RF) interference. Radio frequency pulses from electrical apparatus, flickering fluorescent lamps, and the sparking motor brushes may sometimes be counted by these electronic counters.

Exercises (022):

1. When the machine results do not correspond with the clinical situation, what source of error should be a prime consideration?

2. In the Coulter counter system, what effect does an error in diluting have on the red cell count and hematocrit?

3. How is the MCV, which is automatically computed by the Model S and accessory for the models B, D, F, affected by erroneous dilution?

4. What condition will result if destruction of leukocytes occurs before the lysis of red cells?

5. What conditions may produce false signals or radio-frequency interference yielding counts?

6. In using the electronic counter, why should the total white count in leukemia patients be checked against the slide?

2-3. Microscopic Studies

While working in hematology, you spend much of your time using the microscope. Most of your microscopic work is devoted to the routine differential. Though a clinically vital area, the differential count requires considerable knowledge and often subjective determinations. In Chapters 3 and 4 of this volume we will review the morphology of normal and abnormal cells—both erythrocytes and leukocytes. How well you understand the maturation sequence of cells will necessarily determine how well you perform differentials. However, there are even more basic considerations than identifying cells. We will discuss some of these areas in this section.

023. Indicate the significance of the differential cell count, and identify the particular cell that shows an increase percentage in a given disease.

The Differential. The differential white cell count is done in order to determine the relative number of each type of white cell present in the blood. An integral part of this analysis is an evaluation of atypical leukocytes, normal or abnormal erythrocytes, and the quantity and quality of platelets encountered during the leukocyte count.

Detailed examination of the stained blood smear yields more information than any other single laboratory test. When the complete blood count is performed, the differential should be done last. In this manner, examination of the smear may be used to doublecheck the white cell count, and a very rough estimate of the hemoglobin, hematocrit, and red cell count may be made. The normal values for the differential white count will vary somewhat with age; however, the adult values are shown in table 2-1. Infants and children of preschool age are exceptions.

Diseases Related to White Cell Type. In disease, a specific type of white cell may show an absolute increase in percentage in the blood. The increased cell percentages which are found in the more common diseases are given below.

a. Increase in the number of neutrophils (neutrophilia) may indicate one of the following:

(1) Appendicitis.
(2) Pneumonia.

TABLE 2-1
NORMAL ADULT DIFFERENTIAL VALUES

	Percent
Neutrophilic metamyelocytes	0-1
Neutrophilic bands	3-5
Segmented neutrophils	55-65
Eosinophils	2-4
Basophils	0-1
Lymphocytes	20-35
Monocytes	2-6

(3) Myelogenous leukemia.
(4) Bacterial infection.

b. Increase in the number of eosinophils (eosinophilia) may indicate:
(1) Allergenic reactions.
(2) Allergies.
(3) Asthma.
(4) Scarlet fever.
(5) Parasitic infection.

c. Increase in the number of lymphocytes (lymphocytosis) may be an indication of:
(1) Viral infections.
(2) Whooping cough.
(3) Infectious mononucleosis.
(4) Lymphocytic leukemia.

d. Increase in the number of monocytes (monocytosis) may indicate one of the following:
(1) Tuberculosis.
(2) Brucellosis.
(3) Monocytic leukemia.
(4) Subacute bacterial endocarditis.

e. Increase in the number of basophils (basophilia) may be an indication of:
(1) Chronic granulocytic leukemia.
(2) Hemolytic anemia.
(3) Removal of spleen.
(4) Irradiation (X-ray).
(5) Polycythemia vera.

The importance of the cell differential is evidenced by the information provided in confirming diagnosis of numerous diseases.

Exercises (023):
In exercises 1 through 3, indicate whether each statement is true or false, and correct those that are false.

T F 1. The differential white cell count is done in order to determine the relative number of each type of white cell in the blood.

T F 2. The differential includes checks for atypical leukocytes, normal erythrocytes, and abnormal erythrocytes, but does not include evaluation of the quantity and quality of platelets.

T F 3. When the complete blood count is performed, the differential count should be done first.

In exercises 4 through 14, match each absolute increase of specific white cell types in column B with the disease or condition in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each column B element may be used more than once.

	Column A	*Column B*
____	4. Myelogenous leukemia.	a. Basophilia.
____	5. Appendicitis.	b. Eosinophilia.
____	6. Allergenic reaction.	c. Neutrophilia.
____	7. Viral infections.	d. Lymphocytosis.
____	8. Tuberculosis.	e. Monocytosis.
____	9. Removal of spleen.	
____	10. Pneumonia.	
____	11. Parasitic infection.	
____	12. Infectious mononucleosis.	
____	13. Brucellosis.	
____	14. Irradiation (X-ray).	

024. Indicate whether given statements reflect the correct procedures and techniques for processing blood smears for differential cell counts.

Processing Blood Smears. All routine blood smears should be kept in the hematology section for a period of 1 week. Occasionally, a review of a specific problem slide results in findings that were not originally apparent and reinforces confidence in the laboratory by the medical staff. This practice also adds to the experience and proficiency of the technician.

Slides must be clean and free of oil, grease, lint, or dust to prepare good blood smears. It is best to use new slides, but sometimes it is necessary to clean even new slides in 95 percent alcohol. Blood smears are usually made from a sample of blood from the needle immediately after venipuncture. Otherwise, the sample may clot or dry. A full falling drop of blood is an excessive amount for one glass slide. Approximately half of this amount is ideal. Blood smears should not routinely be made from blood containing an anticoagulant. Anticoagulants distort the cells and alter staining characteristics. Two or occasionally more blood smears should be made initially so that additional slides may be stained without collecting another specimen.

Use a sharp lead pencil or black felt tip marker to write the name of the patient in the thick area of the smear. A properly made blood film covers at least one-half the length of the slide but not more than three-fourths of the total length.

Allow the blood film to air dry completely. As you probably know, you should not blow air on the slide in an effort to enhance drying, because this may distort the cells.

In marked leukopenia, smears can be made from the white cell layer ("buffy coat"). In this case, you must be careful to use all of the buffy coat, and not a selected fraction which may not contain representative cells.

Centrifuge the blood in a Wintrobe hematocrit tube at 500 to 800 r/min for 5 minutes. Remove the buffy coat and make the slide in the usual way.

Slides should be stained within a few hours after they are made. If a delay of more than 3 to 4 hours is necessary, slides should be fixed in 95 percent methanol for 30 minutes. This protects the slides in areas of high humidity. Excess moisture hemolyzes red cells, while white cells become distorted or disintegrate rapidly from bacterial action.

Protect blood slides from insects, because flies and cockroaches will eat the fresh blood. If this is a problem, you should follow the procedure of fixing the slides in methanol and storing them in slide boxes.

Exercises (024):
Identify each true statement, and correct those that are false.

T F 1. All routine blood smears should be kept in the hematology section for 1 year.

T F 2. Blood smears may be routinely made from blood containing an anticoagulant since anticoagulants enhance the staining of the cells.

T F 3. Blowing on the slide to dry it is permissible and may enhance quality of cells.

T F 4. If a delay of more than 3 to 4 hours is necessary, slides should be fixed in 95 percent isopropanol for 30 minutes.

T F 5. Excess moisture tends to hemolyze red cells, and white cells become distorted or disintegrate rapidly from bacterial action.

T F 6. Protect blood slides from insects because flies and cockroaches will eat fresh blood.

T F 7. In marked leukocytosis, smears can be made from the white cell layer, "buffy coat."

T F 8. To obtain cells from the buffy coat, centrifuge the blood in a Wintrobe hematocrit tube at 500 to 800 r/min for 5 minutes.

025. Point out constituents of the stains, staining methods, and techniques for peripheral blood and bone marrow smears.

Stains. Different stains and different techniques are used to stain blood films. Two types of stains are in general use—those which stain fixed cells, and those which will stain living cells (supra-vital stain). The panoptic (differentiating) stains generally used in hematology are Giemsa and Wright's stains. When optimal staining conditions exist, Wright's stain is very satisfactory and easily differentiates cells. Wright's stain consists of methylene blue and eosin dissolved in methyl alcohol. Giemsa stain may be included in the staining solution. The alcohol also fixes the blood film to the slide.

Staining Methods. The staining process may be performed by (1) the staining rack method, (2) the staining jar or "dip" method, or (3) the automatic method.

Staining rack method. When Wright stain is applied by the staining rack method, it should cover the slide, and must not be allowed to remain long enough to evaporate. Wright's buffer should be added to the stain in a small stream without overflowing the slide. Blowing gently on the slide sometimes is recommended if the stain-buffer mixture is not evenly distributed. A green sheen appears if the stain and buffer are properly mixed. The times for staining and buffering should be adjusted with each fresh bottle of stain to give the best results. The stain should be flushed from the slide with distilled water. Wipe excess stain from the undersurface of the slide and air dry the smear.

Very little actual staining takes place during the fixing stage. Most staining occurs during the buffering stage. It is important to add just enough buffer; otherwise, the smear will stain too lightly. Do not blot the smear or place it in the heat of a lamp. Heat darkens the stain and may cause cell distortion.

Staining jar method. This method involves the dipping of the blood smears into several solutions. It is less commonly used. However, the jars containing methanol are quite convenient for fixing of smears before placing in the staining rack.

Automatic method. The automatic method involves the staining of blood smears by an ingenious machine. The Hema-Tek slide stainer is illustrated in figure 2-9. This instrument provides a completely automated method for Wright staining blood smears. The machine holds 25 slides, which are carried by a spiral conveyor down a platform (platen). These slides are

7. What condition may result if the slide is placed in the heat of a lamp?

8. What can happen if slides are not properly aligned between the conveyor spirals of the Hema-Tek slide stainer?

9. The volume control knobs serve what function?

10. Even though the staining times may vary from one laboratory to another, how does the staining time for bone marrow smears compare with that for peripheral smears?

026. Identify the causes of given staining reactions when using the Wright's stain and procedures used to correct these reactions.

Staining Reactions. Microscopically, properly stained red blood cells are buff pink. If the RBCs are blue, this indicates that the stain is too alkaline. With an alkaline stain, the WBCs stain dark and have only fair distinguishing characteristics. However, abnormalities of the RBCs will be masked by the heavy stain. A dark stain may be caused by smears that are too thick, overstaining, evaporation of the stain, a stain or diluent that is alkaline, and alkaline fumes.

If the red blood cells are too red, the stain is too acid. In this situation, the white blood cells (except eosinophilic granules) stain very poorly. The tendency toward acid staining is caused by incomplete drying before staining, insufficient staining, and overdilution of the stain with buffer. Acid staining is also often caused by prolonged washing of the slide after staining, the use of stain or buffer that is acid, and by acid fumes in the laboratory.

As a technician, you should strive for a staining reaction that is neither too alkaline nor too acid in order to acquire optimum distinguishing features for all the cells. If the staining reaction is excessively alkaline, decrease the time of staining or neutralize the stock stain solution with 1 percent acetic acid or 1 percent hydrochloric acid. If the staining reaction is too acid, increase the time of staining or neutralize the stock stain solution with 1 percent potassium bicarbonate, or a weak solution of ammonia water. Check the results on trial slides after the addition of each drop of the acid or alkalizer. A poorly stained smear may be saved by destaining rapidly with 95 percent alcohol, washing quickly in water, then restaining. Most often, it is better to start anew, making fresh stain, buffer, or both. Refer to table 2-2 for a comparison of properly and improperly stained cells.

In some areas, distilled or demineralized water may be used in place of a buffer solution. If acid or alkaline stains result, you should change to a buffer solution prepared with distilled water. Preweighed buffer salt tablets are available commerically, which simplify the preparation of buffers. Table 2-2 describes the various staining reactions.

Exercises (026):

Identify each of the column B items with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

TABLE 2-2
STAINING REACTIONS UNDER VARYING CONDITIONS

Type of Blood Cell or Component	Good Stain	Acid Stain	Alkaline Stain
Erythrocytes	Buff-pink	Bright red or orange	Blue or green
All nuclei	Purple-blue	Pale blue	Dark blue
Eosinophilic granules	Granules red	Brilliant, distinct	Deep gray or blue
Neutrophilic granules	Violet-pink	Pale	Dark, prominent
Lymphocyte cytoplasm	Blue	Pale blue	Gray or lavender

Column A

____ 1. Properly stained erythrocytes.

____ 2. WBCs stain dark.

____ 3. Caused by smears that are too thick, overstaining, evaporation of the stain, and alkaline fumes.

____ 4. RBCs are blue.

____ 5. RBCs are too red.

____ 6. WBCs stain poorly.

____ 7. Caused by incomplete drying before staining, insufficient staining, and overdilution of the stain with buffer.

____ 8. May be caused by prolonged washing.

____ 9. Decrease staining time or neutralize the stock stain solution with 1 percent acetic acid or 1 percent hydrochloric acid.

____ 10. Increase staining time or neutralize stock stain with 1 percent potassium bicarbonate or a weak solution of ammonia water.

____ 11. May be used in place of a buffer solution.

____ 12. Lymphocyte cytoplasm appears pale blue.

____ 13. Neutrophilic granules appear violet-pink.

Column B

a. When staining is acid or excessively acid.

b. Distilled or demineralized water.

c. When staining is alkaline or excessively alkaline.

d. Buff pink.

e. Dark staining.

f. Good stain.

027. Cite morphological and distinctive inclusion characteristics utilized to differentiate white blood cells in the examination of stained blood smears.

Morphological Characteristics. Certain morphological cell inclusion characteristics are utilized to differentiate blood cells. A review of the significant features will promote a better understanding of blood differentials. Cellular characteristics such as relative size, shape, cytoplasmic granulation, nuclearcytoplasmic ratio, configuration, chromatin, or nucleoli are very important.

You will recall that there are two basic parts to each white cell: the nucleus and the cytoplasm. However, if a cell is not readily identified, you should ask yourself these three questions: (1) What is the size of this cell? (2) What are the features of the nucleus? (3) What are the features of the cytoplasm?

The above questions are developed in greater detail, in addition to other significant characteristics in the following discussion.

Nuclear configuration. The nuclear configurations of leukocytes help distinguish these cells. "Round," "oval," "indented," "band," or "segmented" are terms used to describe variations in shape. These normal configurations can be distorted by physical and chemical factors mentioned previously. Some of the leukocytes are so fragile that in thick blood smears their normal configuration may be distorted by the pressure of erythrocytes forced against them. These artifacts should be recognized as such in an intelligent evaluation of blood differentials.

Problems have arisen concerning the differentiation of band neutrophils, but there is a conventional method of differentiating them. The distinction depends only upon nuclear configuration. The precursor of the band cell is the metamyelocyte (juvenile) cell, and the more mature cell is the segmented neutrophil. The nucleus of the metamyelocyte is described as kidney or bean shaped. The nucleus of the segmented neutrophil is divided into lobes that are joined by filaments. Since the band cell lies between these two in its stage of development, any cell that becomes more indented than bean shaped and does not possess filaments must be called a band cell. The only exception would be a neutrophil more mature than a metamyelocyte in which the nucleus is folded upon itself and not completely visible. According to the rule of differential counting, this folded cell is called the more mature cell—a segmented neutrophil.

Inclusion characteristics. In addition to nuclear shape and size, the internal nuclear morphology shows differential inclusions. The chromatin appears finely reticulated in some cells, or as a coarse network, or even clumped, in others. The parachromatin, a lighter staining material beside the chromatin, may be scant or abundant. The appearance of the chromatin and the quantity of parachromatin are utilized to differentiate blood cells. The presence, absence, and number of nucleoli in the nucleus are the most distinctive characteristics of immature nuclei in blood cells.

Size. Size considerations in differentiating blood cells require a defined linear standard. The micrometer (μm) .001 mm is usually used in reference to microscopic dimensions. Ocular micrometers are available through Air Force medical supply channels and are easily calibrated, using a hematocytometer which has standardized dimensions. In routine screening of blood smears, an experienced technician relates the size of a normocytic erythrocyte (7 to 8 micrometers) to the size of the white cell to be differentiated, since erythrocytes are usually present throughout the microscopic field. Finally, it should be understood that personal visual discrimination is an inaccurate gauge of linear measure. Some reference measure should be employed.

Nuclearcytoplasmic ratio. The size ratio of nucleus to cytoplasm (N:C) is a differentiating characteristic. For instance, a cell with a nuclear mass equal to the cytoplasmic mass would have an N:C ratio of 1:1. The total cell mass is usually greater in the more immature cells and decreases as the cell matures. The nuclear mass usually decreases also as the cell matures. Of course, lymphocytes are the exception to this generality.

Shape. The shape of blood cells often depends upon the smear and staining technique. Variations that have no clinical significance may occur from physical and chemical distortions that result from technical error. These variations can be avoided with more careful technique. Each routine smear should be scanned initially to evaluate the smear and stain quality before differential analysis.

Cytoplasmic granulation. Cytoplasmic granulation—neutrophilic, basophilic, or azurophilic—is an important morphological observation. Differences in granule color in Wright stained preparations are caused by the variable dye affinity of specific granules. The intensity of colors and the relative blueness or redness of the erythrocytes may be used to evaluate the quality of the stain. The familiar basophilic (blue), eosinophilic (red), and neutrophilic (pink) granules are quite obvious in routine blood smears. The presence, absence, type, and quantity of granules are characteristic attributes used to differentiate leukocytes.

Exercises (027):

1. What three questions should you ask yourself if a cell is not readily identified?

2. What is the best way to determine cell size?

3. What do we mean if we say that cell has an N:C ratio of 1:2?

4. What happens to the nuclear mass as the cell matures?

5. What type of white cells are exceptions to the generality stated in exercise 4?

6. The shape of the cells often depends upon what two features?

7. From what two possible sources may variations in cells occur that could result in technical error?

8. With reference to granules, what characteristic attributes may be used to differentiate leukocytes?

9. Some leukocytes are so fragile that their normal nuclear configuration may be distorted by what condition?

10. How is a band cell distinguished from the metamyelocyte?

11. How is a band cell distinguished from a neutrophil?

12. What are the most distinctive features of an immature nucleus?

028. Indicate whether given statements correctly reflect the procedures for examination and evaluation of stained blood smears and the estimation of the total white count.

Examination of Stained Blood Smear. Examine a stained blood smear under low power magnification to check the general distribution of cells and stain quality. Select a thin, well-stained area under high power magnification and switch to the oil immersion objective for your differential count. The intracellular morphology previously mentioned for differentiation cannot be observed adequately except with the oil immersion lens. A properly prepared smear should be thin enough so that there are few overlapping red blood cells, uniformly stained, and relatively free of precipitated stain. Normals for a differential count are shown in table 2-1.

Observe the platelets in several oil immersion fields to estimate their number and report as normal, increased, or decreased. The normal is an average of 4 to 8 platelets for every 100 red cells. If they appear significantly decreased, a thrombocyte count or coagulation test may be indicated.

Many times you may find that leukocytes are not evenly distributed over the smear. Monocytes and granular leukocytes tend to clump near the edges of the smear, while lymphocytes remain in the central portion. The cells at the extreme edge of the smear should not be counted. They are likely to be distorted and are very difficult to differentiate.

In routine laboratory work, counting 100 consecutive leukocytes on a stained blood smear is usually sufficient for a clinical workup on a patient. However, if the differential leukocyte count is abnormally high, an additional 100 white cells should be evaluated. If you count 200 cells, you may find that division by 2 for a particular kind of cell results in a decimal figure. You should not report the decimal fraction. In rounding a number, always report the lesser figure for cells that are relatively rare. For example, if you count 5 eosinophils in 200 cells, report 2 instead of 3. Be sure that the total count adds up to 100.

The gradual transition from the metamyelocyte to the band and then to the segmented granulocyte makes precise classification difficult. A good rule to follow is to classify the cell according to the more mature form.

Estimation of the Total White Count from Stained Smear. As previously mentioned, changes in the type, number, and morphology of white blood cells in the peripheral blood are extremely valuable indicators of the presence and cause of disease. In most cases the result of the total white count correlates with quality and quantity of leukocytes noted on the stained blood smears. However, there are some exceptions. Thus, the slide may have to be used as a valuable check on counting methods. An estimation of the WBC counts is suggested in the following:

WBC per hpf	*Total count estimated*
2–4	4,000–7,000
4–6	7,000–10,000
6–10	10,000–13,000
10–20	13,000–18,000

Fragile cells in leukemia may disintegrate in the Coulter counter; therefore, the white count should be estimated from the slide as a check on the Coulter counter result. The estimation of the white count should not be routinely substituted for more accurate and precise counts as a part of a complete blood count.

Exercises (028):

If one of the following statements is correct, mark it true. If it is false, correct it.

T F 1. Excellent intracellular morphology for differentiation can be observed under high power magnification.

T F 2. After observing an average of 4 to 8 platelets per 100 RBCs, the results of the platelet estimation may be reported as decreased.

T F 3. Lymphocytes and granulocytes tend to clump near the edges of the smear, while monocytes remain in the central portion.

T F 4. The cells at the extreme edge of the smear should not be counted because of possible distortion and difficulty in differentiation.

T F 5. If the differential leukocyte count is abnormally high, an additional 100 white cells should be evaluated.

T F 6. If you count 7 eosinophils in 200 cells, report 4 instead of 3 for average per 100 white cells.

T F 7. A good rule is to classify the cell according to the more mature form.

T F 8. A total of 9 WBCs per high power field were counted on a blood smear from a leukemic patient. The estimated total white count would be around 18,000 to 27,000 WBCs.

T F 9. Fragile cells in leukemia may disintegrate in the Coulter counter, requiring an estimated white count from the slide as a check.

T F 10. The estimation of the leukocyte count may be routinely used as a part of a complete blood count.

2-4. Hematocrit and Hemoglobin Studies

The oxygen-carrying capacity of blood can easily be determined in the laboratory by means of either a hemoglobin or hematocrit. In most cases, it is desirable for the clinician know both values, especially in evaluating anemias.

029. Identify the methods used for hemoglobin determination in terms of normal values, principles, techniques, accuracy, sources of error, and applications.

Hemoglobin Measurement Values. The measurement of hemoglobin in the blood is a simple means of aiding in the diagnosis of anemia or polycythemia. It can also aid the physician in other ways, such as in assessing blood loss. It can be done quickly with a small amount of blood.

Because of wide variations in the hemoglobin values of normal individuals, it is impossible to give a single exact normal value. Hemoglobin values are usually expressed in grams/dl of whole blood. Significant variations in normal hemoglobin values exist in different age levels and between males and females. The range of values is presented in table 2-3.

Colorimetric Methods. The colorimetric methods for hemoglobin determination include the direct matching, acid hematin, oxyhemoglobin, and cyanmethemoglobin methods.

Direct matching method. In this method, the color of the blood is compared with a series of colored standards of known quantities of hemoglobin. This method has the advantages of being fast, simple, and convenient. The more accurate procedure employing this method is that of Spencer when compared to Tallquist's and Dare's. A hemoglobinometer using the Spencer procedure is an available stocklisted item, FSN 6630-00-926-6989. This method is practical for field conditions and where very limited laboratory facilities are available. The procedure is a direct matching, but since the blood is primarily hemolyzed by stirring with an applicator stick containing saponin, it may also be considered an oxyhemoglobin method.

Acid hematin method. In this method, blood is mixed with dilute hydrochloric acid, which hemolyzes the RBCs and converts the hemoglobin to a brownish colored solution of acid hematin. The acid hematin solution is then compared with a colored glass standard. Acid hematin methods have the advantages of being fast, simple, and inexpensive. However, the methods are slightly but not grossly inaccurate. An acid hematin method using a photoelectric colorimeter is available. The Sahli-Hellige hemoglobinometer is a stocklisted item, FSN 6630-00-428-1200. The item is practical for field conditions and where very limited laboratory facilities are available.

Oxyhemoglobin method. In this method, blood is mixed with a dilute solution of either sodium carbonate or ammonium hydroxide. The hemoglobin is converted to oxyhemoglobin. Resulting color concentration is measured in the spectrophotometer. This method is fast and accurate. But it has one disadvantage in that if traces of copper are present in the diluting fluid, they may convert oxyhemoglobin to methemoglobin and thus slightly lower the results.

Cyanmethemoglobin method. In this method, a sample of blood is mixed with Drabkin's solution, which contains ferricyanide whereby hemoglobin is changed to methemoglobin. This compound unites with cyanide to form cyanmethemoglobin. Cyanmethemoglobin produces a color which is measured as hemoglobin in a spectrophotometer.

This method is fast and accurate and measures all forms of hemoglobin except sulfhemoglobin. There is a disadvantage in that if Drabkin's solution must be prepared, it is necessary to use a poison, cyanide.

Drabkin's solution must be handled with care. Cyanide salts should be stored in a secure area. Discard all solutions containing cyanide in a free flow of water to minimize contact with other chemicals, especially acids, which will release poisonous fumes. Presently, Drabkin's solution need not be prepared from "scratch," as has previously been the case, but can be obtained through medical supply channels. The item is stocklisted as *Cyanmethemoglobin, Reagent,* 12s, FSN 6505-00-579-9293. Since the prepared Drabkin's is so dilute, the danger of cyanide poison is nil. The cyanmethemoglobin method for hemoglobin determination is the method of choice in most hospital laboratories.

Cyanmethemoglobin is a very stable hemoglobin pigment that does not deteriorate for several years if stored in a refrigerator. Of course, you would not expect to rely on any one standard indefinitely. The availability of commercially prepared stable standards is a distinct advantage of this method.

TABLE 2-3
NORMAL VALUES FOR HEMOGLOBIN
IN DIFFERENT POPULATION GROUPS

Population group	Normal values in gm per 100 ml of whole blood
Infants at birth	18-27
Childhood	10-15
Adult males	14-17
Adult females	12-16

Exercises (029):

Match each column B item with statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

____ 1. 10–15 mg/100 ml of whole blood.
____ 2. 12–16 mg/100 ml of whole blood.
____ 3. The color of the blood is compared with a series of colored standards of known quantities.
____ 4. Used in the direct matching procedure and may also be considered an oxyhemoglobin method.
____ 5. Blood is mixed with dilute hydrochloric acid, which hemolyzes RBCs.
____ 6. May be employed in a field laboratory or where limited laboratory facilities are available.
____ 7. Blood is mixed with a dilute solution of sodium carbonate or ammonium hydroxide.
____ 8. In this method, if traces of copper are present the result can be lowered.
____ 9. Contains poisons: ferricyanide and cyanide.
____ 10. In this method, iron is oxydized by the ferricyanide.
____ 11. All forms of hemoglobin are measured except sulfhemoglobin.
____ 12. The method of choice for hemoglobin determination in most laboratories.

Column B

a. Cyanmethemoglobin method.
b. Oxyhemoglobin method.
c. Acid hematin method.
d. Direct matching method.
e. Hemoglobinometer, Spencer procedure.
f. Hemoglobinometer, Sahli-Hellige.
g. Hemoglobin values—adult females.
h. Hemoglobin values—childhood.
i. Drabkin's reagent.

030. State standard procedures for hemoglobin determinations in terms of techniques, reagents, automated instruments, and sources of error.

Procedures for Hemoglobin Determination. The cyanmethemoglobin method is used by most laboratories for reporting manual hemoglobin determinations. It is considered the method of choice because (a) the cyanmethemoglobin is stable in dilute solutions, (b) all common hemoglobin derivatives are measured, (c) certified standards are available, and (d) the spectral curve is such as to allow the use of various kinds of spectrophotometers.

Manual procedure. A manual procedure is outlined in AFM 160-51, *Clinical Laboratory Procedures—Hematology.* The solution must be crystal clear before the unknown sample is read. Any turbidity present will give an erroneously high result. A sample of blood that is overoxalated will not affect the hemoglobin. Cloudiness may be due to:

a. An extremely high white cell count. In these cases, centrifuge the mixture and use the supernatant.

b. Hemoglobin S and hemoglobin C. In this situation, dilute the mixture 1:1 with distilled water, read on the spectrophotometer, and multiply the result by 2.

c. Abnormal globulins. If determined as such, add 0.1 g of potassium carbonate to the test solution.

d. Lipemic blood.

Sources of errors. Sources of error include the following: improperly calibrated pipettes, unclean cuvettes and pipettes, instrument errors (for example, line voltage fluctuations in the spectrophotometer, loose electrical connections, faulty wavelength calibration); improper dilutions, unmatched cuvettes, and improperly prepared or deteriorated Drabkin's solution. If this solution is stored in an opaque container out of direct sunlight, it remains stable at room temperature for several weeks. You should pay particular attention to the loss of color in the blank, since it is exposed continuously to light. A fresh Drabkin blank should be read spectrophotometrically against a water blank. Then subsequent readings during the day will determine whether the Drabkin blank has faded and thus resulted in falsely elevated readings of the unknown.

Spectrophotometer methods—other significant sources of error. For current methods employing spectrophotometry, the probable overall error of hemoglobins should be less than ±0.5 gm percent. A carefully measured manual dilution should have confidence limits of no more than ±0.3 gm percent (2 SD). Pipettes that are obtained through regular supply channels cannot be assumed to be accurate. They must be checked for accuracy. A simple way to do this is to perform a hemoglobin determination on a known sample of blood with each new pipette. If the result is out of range, repeat the procedure until you are certain about the pipette. Acceptable pipettes may be marked in some way to identify them. Technicians must not use new pipettes that have not been checked for accuracy. Experience has shown that some new pipettes will cause results to be in error by as much as 0.75 to 1.0 gram percent of hemoglobin. Pipetting errors usually affect test results significantly. Human variation, as well as calibration differences, is commonly the cause of pipetting errors.

To reduce the problem of technician fatigue, variation in cleanliness of pipettes, and other recurring difficulties, various automated instruments have been developed.

The detailed procedures for standardizing spectrophotometers and determining hemoglobin curves are supplied with each package of hemoglobin standard.

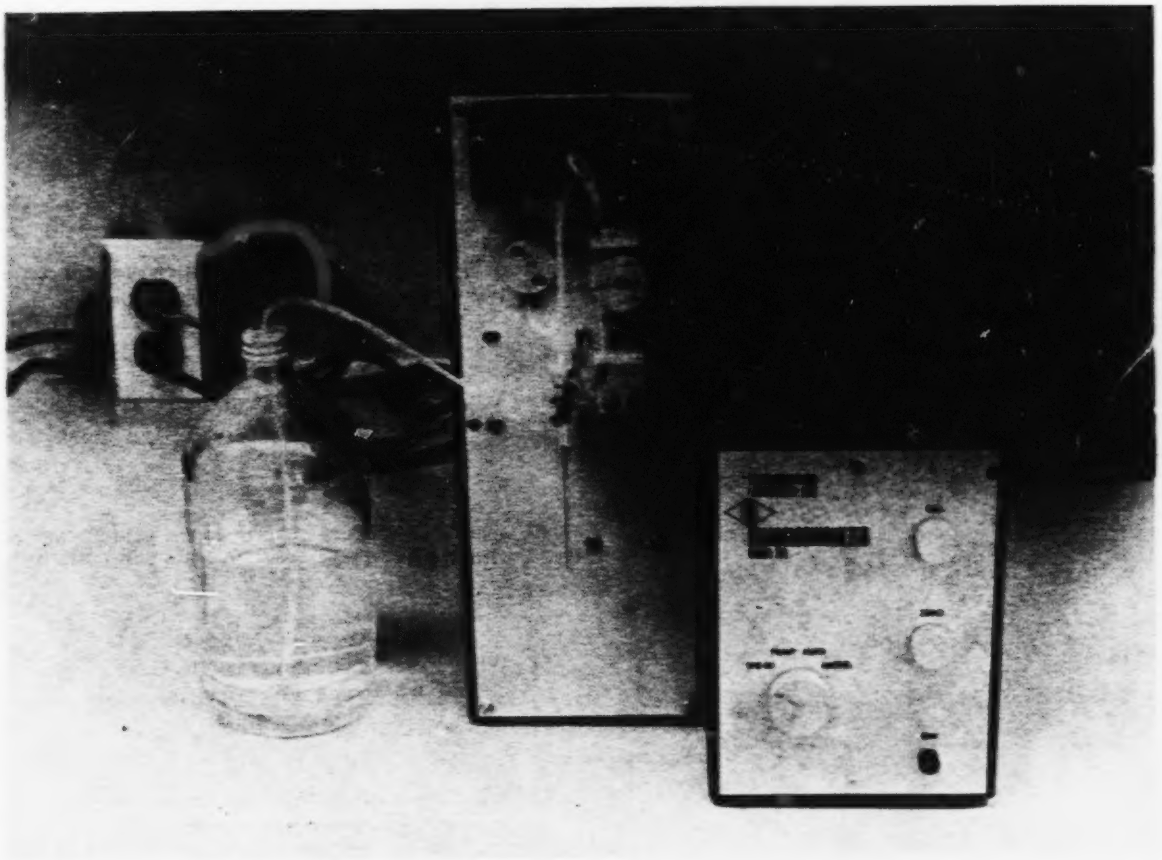


Figure 2-10. Flow-through hemoglobinometer.

Automated procedures. Many automated instruments (hemoglobinometers) for hemoglobin determination, such as shown in figure 2-10, are currently available that utilize cyanmethemoglobin or oxyhemoglobin methods. They are used in conjunction with automatic diluters, as shown in figure 2-11. Some diluters are incorporated in the hemoglobinometers. The diluters in conjunction with these hemoglobinometers are strongly recommended when necessary because of their speed, accuracy, and high precision.

A flow-through hemoglobinometer is pictured in figure 2-10. Operation of the machine is simple. Prior to actually testing a specimen, the machine must be set, using a baseline saline solution. This is done by running saline through the machine and adjusting to zero with the zero control. Next, the machine is calibrated, using an amaranth dye solution. The machine is adjusted to 15 grams/dl with this calibration solution. The machine is now ready to accept the actual specimen. An internal pump sucks the specimen up through the long slender sampler on the front of the machine. An aliquot of a hemolyzing solution is then pumped from the reservoir (on the left). The specimen is mixed, hemolyzed, and passed through a filtered cuvette for reading. The reading goes to an analog computer, which converts it into a numerical reading that is displayed on the digital readout register. In principle, this method measures the three naturally occurring hemoglobin pigments (oxyhemoglobin, reduce hemoglobin, and carboxyhemoglobin). It is not a modification of the standard cyanmethemoglobin procedure. Repetitive dilutions may be made easily and accurately with an instrument similar to the one illustrated in figure 2-11. The concept of an automatic diluter involves (a) the sampling, or "obtaining a measured amount" from a larger volume, and (b) the dilution of the sample. The automatic diluter is essentially two syringes that operate in a systematic manner. One syringe measures and draws the sample; the other syringe measures the diluent. Both of the syringes can be set to draw a metered amount repeatedly within an allowable range of error. Using the automatic diluter, the technician can "pipette" with increased reproducibility. Do not forget, however, that reproducibility (precision) and accuracy are not synonymous terms.

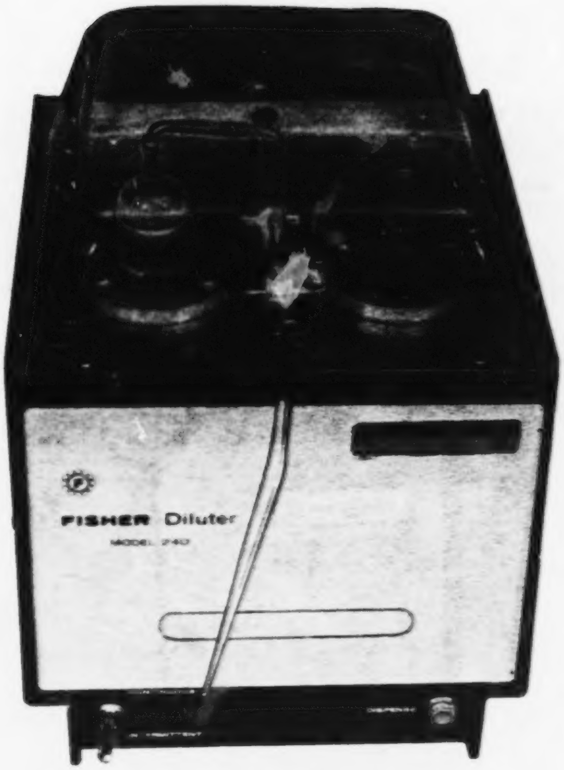


Figure 2-11. Automatic diluter.

Exercises (030):

1. In the manual procedure for hemoglobin determination, what should be the condition of the unknown sample?

2. How would turbidity affect the result of the sample?

3. How would overoxalated blood affect the result of the hemoglobin determination?

4. When cloudiness in the sample is a result of a high white cell count, how can the situation be corrected?

5. When cloudiness in the sample is a result of hemoglobins S and C, what correction must be made?

6. Under what storage conditions would Drabkin's solution remain stable for several weeks?

7. List three sources of instrument error.

8. What is the maximum probable error for spectrophotometric hemoglobin determination?

9. What is the most significant contributing factor to hemoglobin errors by the manual method?

10. List three sources of error in the cyanmethemoglobin procedure.

11. What are three advantages in the use of automatic diluters with hemoglobinometers?

12. What types of hemoglobin are measured by the hemoglobinometer shown in the text?

13. How does the use of the automatic diluter aid in assuring good quality control?

031. Indicate whether given statements correctly reflect the specific characteristics of the three most significant hemoglobin pigments.

Abnormal Hemoglobin Pigments. The three abnormal hemoglobin pigments of great significance are carboxyhemoglobin, methemoglobin, and sulfhemoglobin. These "inactive" forms of hemoglobin are not converted to oxyhemoglobin by an oxyhemoglobin method. These abnormal hemoglobin pigments will be discussed below.

Carboxyhemoglobin. This type of hemoglobin is formed by the combination of hemoglobin with carbon monoxide. The formation of carboxyhemoglobin is reversible. It may be found in the blood of tobacco smokers in concentrations of 2 to 10 percent.

Methemoglobin. This type of hemoglobin is found in the condition in which the ferrous ion has been oxidized to the ferric state and is thus incapable of combining with or transporting the oxygen molecule, which is replaced by an hydroxyl radical. The formation of methemoglobin is reversible and is normally present in the blood in concentrations of 1 to 2 percent.

Sulfhemoglobin. This type is not normally found in blood. If present, its formation is irreversible, and it remains for the life of the carrier cell. It is incapable of transporting oxygen. Its exact nature is not known, and it is thought to be formed by action of certain drugs and chemicals such as sulfonamides and aromatic amines.

Exercises (031):
Indicate whether each of the following statements is true or false, and correct those that are false.

T F 1. The three abnormal hemoglobin pigments may be easily converted by the oxyhemoglobin methods.

T F 2. Carboxyhemoglobin is formed by the combination of hemoglobin and carbon dioxide.

T F 3. The formation of carboxyhemoglobin is reversible.

T F 4. Carboxyhemoglobin is found in tobacco smokers in concentrations of 0.2 to 1.0 percent.

T F 5. Methemoglobin is found in the condition in which oxygen has combined with carbon dioxide.

T F 6. Methemoglobin formation is reversible and is normally in concentrations of 10 to 20 percent.

T F 7. Sulfhemoglobin is normally found in blood and its formation is irreversible.

T F 8. Sulfhemoglobin remains for the life of the cell and is incapable of transporting oxygen.

T F 9. Sulfhemoglobin is thought to be formed by action of certain drugs and chemicals such as sulfonamides and aromatic amines.

032. Indicate whether given statements correctly reflect the processing technique and sources of error in hematocrit determinations.

Hematocrit. The hematocrit procedure is so widely used that only a few comments are necessary. This is a very important test. The relative merits of the hematocrit (Hct) versus a hemoglobin (Hgb) determination have long been argued, but as medical laboratory technicians, we are concerned with accuracy and sources of error more often than which test is to be performed. Allow capillary, or well mixed anticoagulated blood, to enter two capillary hematocrit tubes until they are approximately two-thirds filled with blood. During this process, the presence of air bubbles denotes poor technique, but the test results will be unaffected. A commercial plastic sealing material is preferred to modeling clay or heat sealing of the capillary tube. Tubes are centrifuged for 5 minutes. Remove the tubes as soon as the centrifuge has stopped spinning. Read the results of both hematocrits, using the microhematocrit tube reading device. If microhematocrits cannot be read promptly after centrifugation, the capillary tubes must be properly identified and placed in a vertical position or centrifuged again. Slanting of the cell layer will occur if tubes are left in a horizontal position for more than 30 minutes. The results of both hematocrit tubes should agree within ±2 percent. If they do not agree, repeat the test.

Sources of Errors. Sources of errors in the hematocrit determination may be separated into three kinds: centrifugation, sample, and technical.

Centrifugation. Inadequate centrifugation of hematocrit tubes or allowing the tubes to stand too long after centrifugation will give falsely elevated readings. In order to obtain the maximum red cell packing, the time and speed of centrifugation are extremely important. In addition, a worn centrifuge may easily result in falsely elevated hematocrit readings, especially if the motor speed is decreased due to worn brushes or other mechanical failure. Generally speaking, it is much easier to underpack the cells than it is to overpack them.

Sample. Prolonged stasis resulting from constriction with a tourniquet for 1 minute or more can result in a falsely high hematocrit of from 2.5 to 5 percent. This error is also applicable to hemoglobin and cell counts. Overoxalated blood will give falsely low hematocrit readings due to the red blood cell shrinkage.

Technical. Incomplete sealing of the hematocrit tubes will generally give erroneously low results, because as the tube spins, there is a greater loss of red blood cells than of plasma. In addition, improper order of identification; misreading of the red cell level by including the buffy coat; squeezing the finger during puncture, causing dilution of specimen with tissue fluid; and failure to mix the blood adequately before sampling may result in unnecessary technical errors.

Automated hematocrit. Values of the hematocrit can be determined by automated sequential analyzers and counters, but are usually calculated values. Regardless of what method is used, quality control of the procedure is essential if you are to perform an accurate test. If you observe the sources of error previously mentioned and check test results using the statistical methods discussed in the next section, a significant number of errors will be avoided.

Exercises (032):
Indicate whether each of the following statements is true or false, and correct those that are false.

T F 1. The presence of air bubbles in the hematocrit tubes during filling will adversely affect the results of the test.

T F 2. A plastic sealing material is preferred to modeling clay or heat sealing of the capillary tube.

T F 3. If microhematocrit tubes cannot be read promptly after centrifugation, they must be properly identified and placed in a slanting position which will not affect the cell layer.

T F 4. The results of both hematocrit tubes should agree within ± 5 percent.

T F 5. Allowing the hematocrit tubes to stand too long after centrifugation will give falsely lowered results.

T F 6. To obtain the maximum packing of red cells, the time and speed of centrifugation are very important.

T F 7. Prolonged stasis for 1 minute or more can cause a falsely elevated hematocrit from 2.5 to 5 percent.

T F 8. Overoxalated blood will give falsely elevated hematocrit readings due to the swelling of red blood cells.

T F 9. Incomplete sealing of the hematocrit tubes, through centrifugation, can give erroneously high results.

T F 10. The value of the hematocrit from automated sequential analyzers and counters is a calculated value.

2-5. Statistical Techniques in the Calculation of Laboratory Results

Quality control has been established as an integral part of the clinical laboratory operation. In clinical chemistry, numerous pure standards, lyophilized control specimens, and statistical evaluations are maintained and utilized in the attainment of quality production through adequate control.

Until recently, the statistical evaluation of hematology procedures was generally neglected. Of course, most hematology laboratories have insisted upon the use of stable hemoglobin standards and spectrophotometric checks of hemoglobin calibration. They also require the utilization of accurate pipettes (± 1 percent error) and precise pipetting and dilution work. However, more often than not, the quality of hematology procedures has depended upon the integrity and ability of the individual technician. This may be flattering to the technician, but it is not quality control. Technicians cannot, and should not, be expected to operate with machinelike precision.

Exact reproduction of tests is not attainable, and variation in results is a demonstrated fact. The ultimate purpose of quality control in the laboratory is to understand this variation so that confidence limits may be established, erroneous results detected, and appropriate corrective action initiated to rectify any *significant* discrepancy. An adequate quality control system will indicate the degree of confidence in the test and provide ample warning when a procedure is "out of control."

033. Indicate whether given statements correctly reflect the procedures for calculation of standard deviation and preparation of quality control charts in hematology.

Determining Quality Control in Hematology. As you recall from your study of quality control in chemistry, the statistician employs the term "standard deviation" to evaluate variation. In these terms, a statistician might declare that any variation greater than two standard deviations would be "out of control." The probability of any variation greater than two standard deviations (S.D.) is 5.0 in 100.

The approach to control or statistical evaluation of hematology is to employ the S.D. value as a tool. As you may recall from your previous study of quality control, algebraically, standard deviation is the square root of the sum of the squared difference from the mean divided by the total number of values less 1. It is usually expressed as:

$$\text{S.D.} = \sqrt{\frac{\epsilon d^2}{n-1}}$$

where:

d = difference between each value and the mean.
ϵd^2 = sum of the squared difference from the average.
n = the total number of determinations performed.
(N-1 is used only when $N<30$)

For some purposes, it is convenient to express standard deviation as a percentage of the mean. This is especially applicable to the control of counts. When standard deviation is expressed as a percentage of the mean, the value is known as the coefficient of variation.

$$\text{C.V.} = 100 \times \frac{\text{standard deviation}}{\text{mean}}$$

You undoubtedly recall the meaning of these terms from your study of clinical chemistry.

Quality control RBCs and WBCs are commercially available. However, duplicate counts can be used to provide a reference control for red and white cell counts.

Comparative studies of leukocyte counts have been performed by two common automatic instruments with counts made with the hemacytometer. Instrument variation for both normal and abnormal counts was less than half the variation experienced with a hemacytometer. One coefficient of variation represented 2.3 percent with the instruments and 6.5 percent with the hemacytometer. A daily log should be kept for all control values and a coefficient of variation computed for each cell count procedure. In this way daily changes in a laboratory's level of performance can be detected and corrected. It is important that the coefficient of variation be representative of most of the equipment, personnel, and variable laboratory conditions employed in the daily routine.

Calculation of Standard Deviation. Normal and abnormal controls are available containing stable values in seven parameters: RBC, WBC, Hct, Hgb, MCV, MCH and MCHC. The control should be thoroughly mixed by repeated inversion. The average value and standard deviation for each measure resulting from a daily analysis for 30 consecutive days should be computed as shown by the example for hemoglobin in figure 2-12. Since sufficient detail of the standard deviation had been explained in CDC 90411, briefly review the example in figure 2-12 and note the average value and standard deviation for the hemoglobin control pool number 4:

a. The values obtained from daily analyses of the control are recorded in column A.

b. The values from column A are totaled and recorded in block B.

c. The average of column A is obtained by dividing the total (block B) by 30, or the number of times the control was analyzed. The average is recorded in block C.

d. The individual differences of each test result from the average in block C is computed and recorded in column D.

e. The square of each individual difference is recorded in column E.

f. The total of column E is recorded in block F.

g. The standard deviation is calculated as shown in block G. The square roots may be conveniently obtained from a calculator, a table of square roots, or a slide rule.

h. The acceptable range for the control is based upon ±2 standard deviations in block H. Some laboratories may prefer to convert this to the coefficient of variation.

i. The control limits for the test procedure are recorded under block I. The upper limit is determined by adding two standard deviations to the average value.

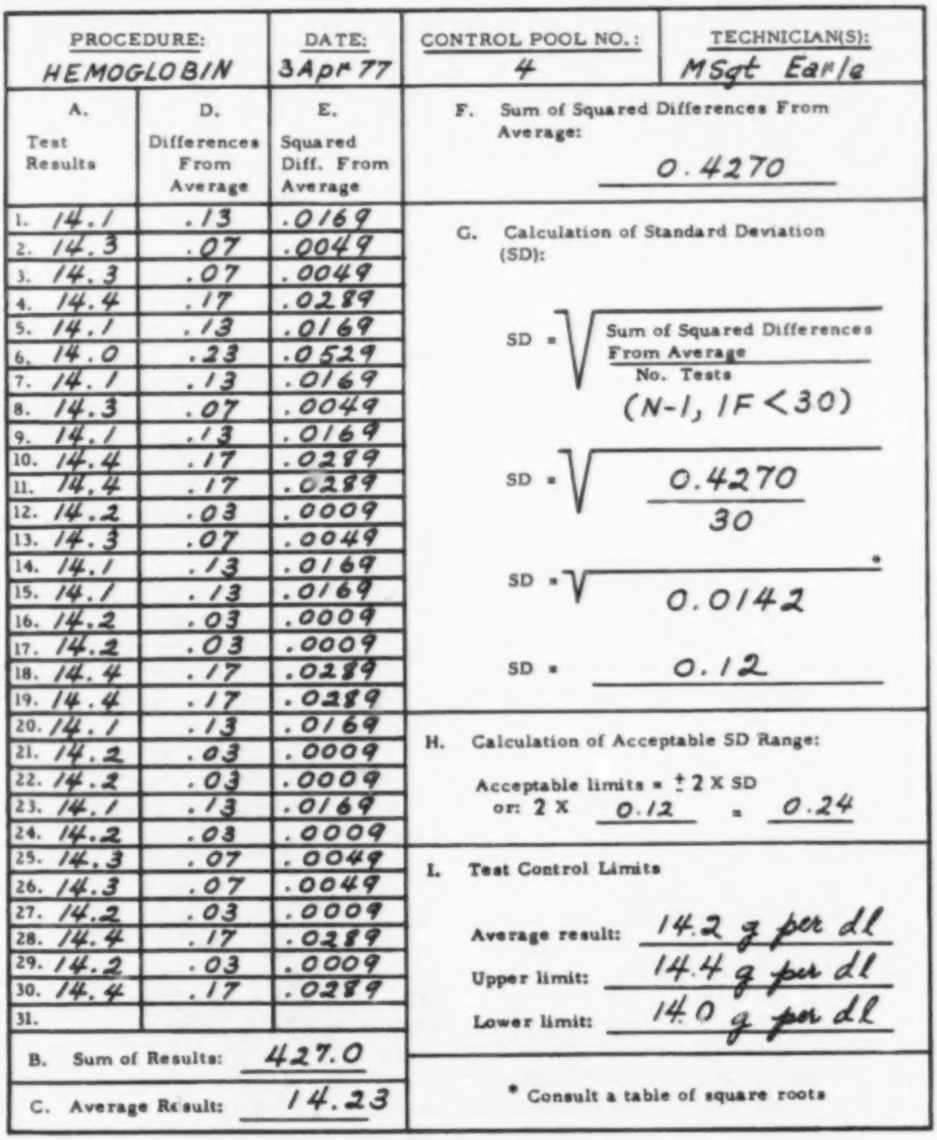
Preparation of Quality Control Charts. When control limits for various hematology procedures have been established, quality control charts should be prepared reflecting the ±2 standard deviation range for each test as shown in the example of a quality control chart in figure 2-13. Let's briefly review this chart:

a. A vertical scale of appropriate test unit values is prepared on the left border of a sheet of graph paper. The scale should extend symmetrically above and below the average value, adequately encompassing ±2 standard deviation values.

b. A solid black line extending from the average value completely across the chart is drawn and labeled "average" at the right border.

c. Similarly, lines representing ±2 standard deviations (SD) are drawn in parallel to the average line. These lines are appropriately labeled ±2 SD.

d. The days of the month are recorded across the bottom of the graph, using two squares for each day.



PROCEDURE:	DATE:	CONTROL POOL NO.:	TECHNICIAN(S):
HEMOGLOBIN	3Apr 77	4	MSgt Earle

A. Test Results	D. Differences From Average	E. Squared Diff. From Average
1. 14.1	.13	.0169
2. 14.3	.07	.0049
3. 14.3	.07	.0049
4. 14.4	.17	.0289
5. 14.1	.13	.0169
6. 14.0	.23	.0529
7. 14.1	.13	.0169
8. 14.3	.07	.0049
9. 14.1	.13	.0169
10. 14.4	.17	.0289
11. 14.4	.17	.0289
12. 14.2	.03	.0009
13. 14.3	.07	.0049
14. 14.1	.13	.0169
15. 14.1	.13	.0169
16. 14.2	.03	.0009
17. 14.2	.03	.0009
18. 14.4	.17	.0289
19. 14.4	.17	.0289
20. 14.1	.13	.0169
21. 14.2	.03	.0009
22. 14.2	.03	.0009
23. 14.1	.13	.0169
24. 14.2	.03	.0009
25. 14.3	.07	.0049
26. 14.3	.07	.0049
27. 14.2	.03	.0009
28. 14.4	.17	.0289
29. 14.2	.03	.0009
30. 14.4	.17	.0289
31.		

B. Sum of Results: 427.0

C. Average Result: 14.23

F. Sum of Squared Differences From Average: 0.4270

G. Calculation of Standard Deviation (SD):

$$SD = \sqrt{\frac{\text{Sum of Squared Differences From Average}}{\text{No. Tests}}}$$

(N-1, IF <30)

$$SD = \sqrt{\frac{0.4270}{30}}$$

$$SD = \sqrt{0.0142}\ ^{*}$$

SD = 0.12

H. Calculation of Acceptable SD Range:

Acceptable limits = ± 2 X SD
or: 2 X 0.12 = 0.24

I. Test Control Limits

Average result: 14.2 g per dl
Upper limit: 14.4 g per dl
Lower limit: 14.0 g per dl

* Consult a table of square roots

Figure 2-12. Example of a monthly ±2 standard deviation calculation.

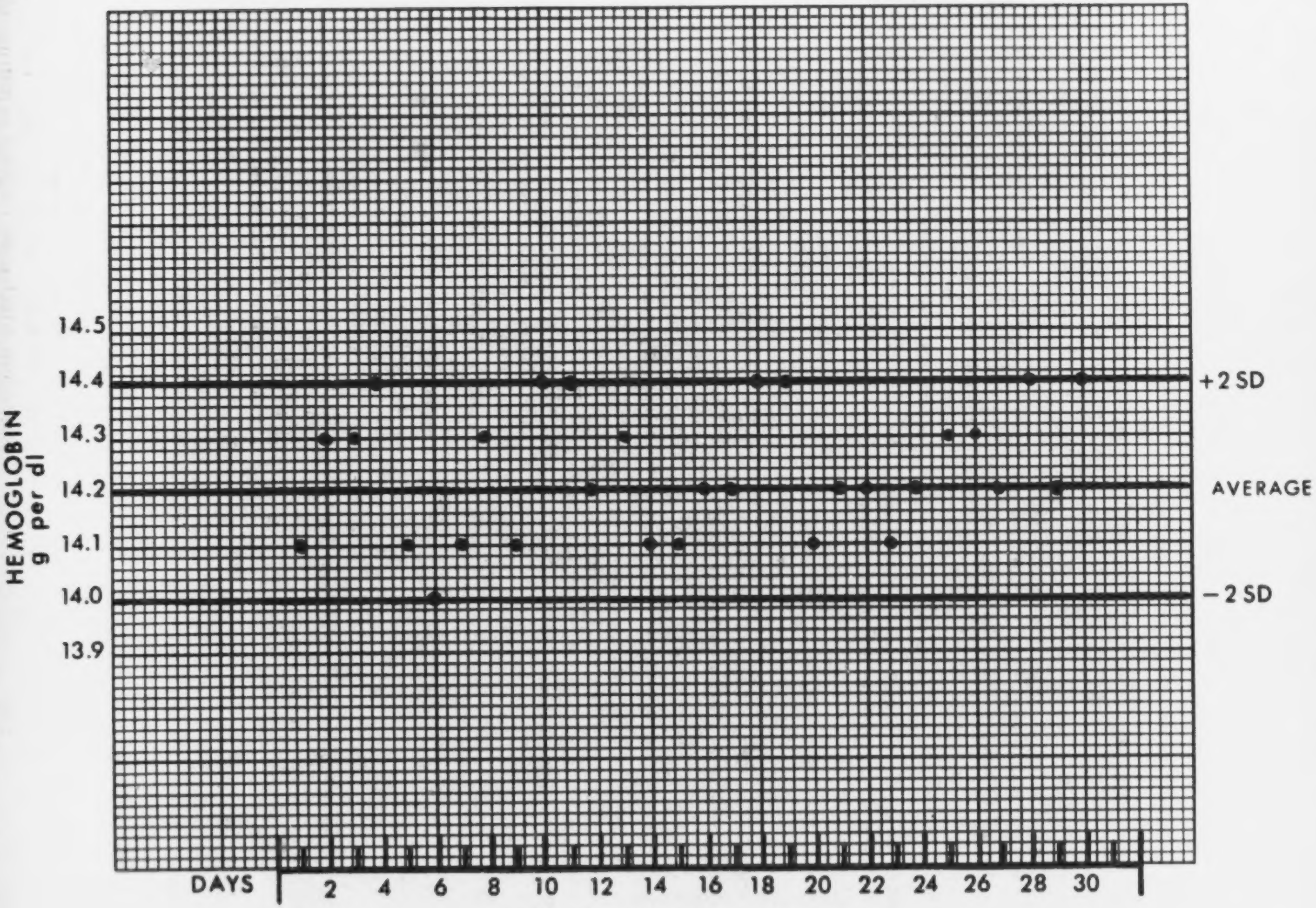


Figure 2-13. Example of a quality control chart.

e. The chart should be labeled with the name of the analysis, the control number, and the month for which the control is to be used in monitoring the test.

Exercises (033):
If one of the following statements is correct, mark it true; if it is false, correct it.

T F 1. The probability of any variation greater than two standard deviations (SD) is 5.0 in 100.

T F 2. The capital letter "N" is used in the standard deviation formula when the total number of determinations performed is less than 30.

T F 3. When the standard deviation is expressed as a percentage of the median, the value is known as the coefficient of variation.

T F 4. Duplicate counts can be used to provide a reference control for red and white cell counts.

T F 5. Comparative studies of leukocyte counts performed indicated a C.V. of 2.3 percent with the automatic instruments and 6.5 percent with the hemacytometer.

T F 6. The differences from the average are entered on the quality control chart and squared.

T F 7. The upper and lower limits are determined by adding ±3 standard deviations.

T F 8. From the example given in figure 2-12, and using the quality control chart in figure 2-13, a hemoglobin value of 14.5 g/dl will still represent +2 SD.

034. From a given list of procedures about quality control application in hematology, identify the true statements and explain why the others are not correct.

Quality Control Application. Procedures for quality control application should be followed wherever applicable and are given below:

(1) On the morning of each duty day, allow a control to warm up to room temperature.

(2) After thorough mixing, analyze a control sample along with one or more clinical specimens with each applicable procedure. Both the control sample and patient specimen must be processed by the same technician under the same conditions for any given procedure.

(3) If the control value falls within ±2 standard deviations (SD), report the results of patient specimen analysis.

(4) Record the control sample result on the appropriate quality control chart for the day concerned, as shown in figure 2-13.

(5) If the control value is outside the ±2 limits, recheck test calculations. If this is not the case, repeat the procedure on all specimens, including a new control sample. If the control sample falls within ±2 SD on the second run and clinical specimen values remain largely unchanged, this probably indicates improper handling of the control sample during the first run.

(6) If the second control analysis is out of limit, a basic flaw in the test should be suspected. Carefully check for possible causes such as deteriorated reagents, improper pH, inaccurate temperature, and defective instrumentation.

(7) Report clinical results obtained with an accurate control reading after correction of the test discrepancy. Occasionally this may necessitate recollection of specimens from patients.

(8) Keep a permanent record of all control sample values falling outside ±2 SD in the day-to-day performance of procedures. Enter the cause, if determined, and corrective action taken for each procedure concerned. This data may prove useful in troubleshooting future analytic problems. Record all "out-of-limit" results on quality control charts, since these values should be used in calculating monthly standard deviations as described below.

(9) When 1 month has elapsed, inspect the quality control charts (figure 2-13) for the various methods. Calculate the monthly standard deviation for each procedure that exhibited more or less equal distribution of control values above and below the mean. This is accomplished using the chart values accumulated during the month's run.

(10) Prepare new quality control charts reflecting the adjusted ±2 SD range computed in step (9).

(11) If monthly result trends of any procedure indicate consistent progression toward one control limit or the other, restudy the method. Following any corrections in technique, reagents, or instrumentation, redetermine the ±2 SD limits on a new series of 30 daily control analyses.

(12) Continue analyzing the control in conjunction with clinical specimens, and record control results on

the new quality control charts serving the new month.

(13) In the manner described above, prepare quality control charts for each successive month, always using ±2 SD ranges computed from control sample values of the previous month. In actual practice, this should progressively narrow the acceptable range of analytic error for each procedure.

Exercises (034):
Identify each true statement, and explain why the others are false.

T F 1. Warming of control to room temperature is not necessary.

T F 2. Both the control sample and patient specimen must be processed by the same technician under the same conditions.

T F 3. Patient results are reported if the controls fall within ±3 standard deviations.

T F 4. If the control sample falls within ±2 standard deviations on the second run and clinical specimen values remain largely unchanged, this probably indicates improper handling of the control sample during the first run.

T F 5. If the second control analysis is out of limit, a basic flaw of the control should be suspected.

T F 6. Keeping a permanent record of all control sample values falling outside ±2 SD in the day-to-day performance is not necessary and time consuming.

T F 7. A new series of 30 daily control analyses should be redetermined following any corrections in technique, reagents, or instrumentation.

CHAPTER 3

Erythrocyte Studies

IN THIS CHAPTER we will consider some important aspects of red blood cell studies. First, we will review the normal maturation sequence of erythrocytes and describe some of the variations in size, shape, and hemoglobin content which may be found. Second, we will examine the more basic phenomena of erythrocyte production and destruction. Concluding this chapter is a brief résumé of techniques peculiar to the study of erythrocytes that were not included in Chapter 2.

Red cells can develop qualitative and quantitative variations. Purely qualitative variations include differences in size, shape, internal structure, and type of hemoglobin. Quantitative variations include a decrease or an increase in the numbers of erythrocytes. A qualitative or quantitative change in erythrocytes may affect body metabolism by altering the oxygen-carrying capacity of the red blood cells. Anemia may result if impairment of the oxygen-carrying capacity is severe.

In the production and development of red blood cells (erythropoiesis), these cells undergo a gradation of morphological changes. We study the normal complete cell maturation in bone marrow and peripheral blood to better differentiate abnormal cells, including young forms in the peripheral blood.

3-1. Morphology of Erythrocytes

In maturing, erythrocytes develop a biconcave disk shape which facilitates oxygen exchange of the cell by increasing the surface area of the red cell. The amount of hemoglobin in a blood sample will give us an idea of the oxygen-carrying capacity of the blood. We must correlate this test with an evaluation of the morphology of the erythrocytes. The size, shape, and a rough estimation of the hemoglobin content of red blood cells may be checked by microscopic examination of a stained blood smear. More objective studies are necessary to accurately evaluate the quantitative and measure qualitative variations which occur in normal erythrocytes. The erythrocytes contain hemoglobin, an iron-porphyrin ring compound, which readily takes up and liberates oxygen. Without the hemoglobin transport system, oxygen could not be carried in sufficient quantity by the blood to maintain body metabolism, and death would result.

035. Identify the erythrocytes in their normal maturation sequence in terms of their size, shape, and nuclear and cytoplasmic maturation.

Normal Maturation Sequence. Each of the major groups of cells in the rubricytic series is shown in foldout 1 in the back of this volume. Study each cell carefully as it is discussed. Terms used in Air Force laboratories to describe red blood cells are those recommended by the American Society of Clinical Pathologists and the American Medical Association. The cell descriptions are based on the appearance of well-prepared Wright stained smears of normal blood or bone marrow. The terms with some of their synonyms are as follows:

ASCP Terminology	*Synonyms*
Rubriblast	Pronormoblast
Prorubricyte	Basophilic normoblast
Rubricyte	Polychromatic or polychromatophilic normoblast
Metarubricyte	Orthochromic or orthochromatophilic normoblast
Reticulocyte	---
Erythrocyte	Normocyte

Rubriblast. The rubriblast is a moderately large cell 12 to 15 micrometers in diameter. It has a larger, more rounded, and more centrally located nucleus than do other blast cells. The N:C ratio is about 8:1, so the amount of cytoplasm is described as scant compared to the nucleus. In the earliest forms, the cytoplasm stains light blue, but in the predominant, more mature cells, the cytoplasm stains more darkly with a pink cast, described as royal blue. The cytoplasm is granule free and limited to a thin rim around the nucleus. There is no evidence of hemoglobin formation (polychromatophilia) in the cytoplasm. The nuclear chromatin is finely reticulated in a close, homogeneous, mesh network with sparse, indistinct parachromatin evident. One to two nucleoli are present; however, they may not be clearly visible. If the nucleoli are distinct, they differentiate the rubriblast from the prorubricyte.

Prorubricyte. The prorubricyte is slightly smaller (10 to 15 micrometers) than the rubriblast and contains a round, slightly eccentric (off-centered) nucleus. The chromatin is coarse and clumped with distinct parachromatin, but nucleoli are not present. The cytoplasm of the prorubricyte is deeply basophilic and stains royal blue. Cytoplasmic granules are not present, and there is no polychromatophilia. The unique chromatin pattern, absence of nucleoli, and intense basophilia of the cytoplasm are the most differentiating characteristics.

Rubricyte. The rubricyte is smaller than the prorubricyte (8 to 12 micrometers) with a round, eccentric, dark-staining nucleus that is smaller than the nucleus of the prorubricyte. The chromatin material is coarse and granular with distinct parachromatin, sometimes described as a spoke-wheel pattern. No nucleoli are present. The cytoplasm of the rubricyte is polychromatophilic (blue-pink or grey) due to the first appearance of hemoglobin. There are no cytoplasmic granules. The unique chromatin pattern of the nucleus and cytoplasmic polychromatophilia are the most distinctive characteristics of the rubricyte.

Metarubricyte. The metarubricyte is smaller (7 to 10 micrometers in diameter) than the cells in the erythrocyte developmental series thus far discussed. The cell has a small, round or sometimes bizarre (bilobed, clover leaf, etc.) nucleus which stains intensely basophilic (blue-black). The chromatin is homogeneously dense with no parachromatin. The cytoplasm is orthochromatic (buff-pink), with hemoglobin present in full amount. There are no granules present. The orthochromatic cytoplasm (loss of diffuse basophilia) and homogeneously intense, basophilic nucleus are the most characteristic features of the metarubricyte.

Reticulocyte. The reticulocyte is the immediate precursor of the mature erythrocyte. Normally, in adults, 0.5 to 1.5 percent of all erythrocytes are reticulocytes. When there is an increase in erythropoiesis, there is an increase in these cells. This proliferation occurs following administration of iron in iron deficiency anemias and during the course of other anemias. The percentage of reticulocytes is normally higher in the newborn, ranging from 2.5 to 6.5 percent, but falls to the normal adult range by the end of 2 weeks. Reticulocytes are slightly macrocytic in comparison to other erythrocytes from the same specimen. However, the reticulum (basophilic substance from immature precursors) is not demonstrable in Wright stained preparations. Supravital stains such as new methylene blue must be used to observe reticulocytes. Supravital staining for reticulocytes distinguishes these erythrocytes from those with diffuse basophilia (polychromasia) and basophilic stippling (punctate basophilia), which are both demonstrable with Wright stain.

Whereas reticulocytes are normal in peripheral blood, diffuse basophilia and basophilic stippling are not normally found in erythrocytes except in bone marrow. As you can see from the foregoing descriptions, these terms (reticulocyte, diffuse basophilia, and basophilic stippling) are not synonymous.

Diffuse basophilia. Diffuse basophilia in an RBC is ordinarily caused by residual basophilic substance from precursors. As the term indicates, the basophilia (blue cast) is homogeneously distributed throughout the erythrocyte. These cells are often seen in abnormal peripheral smears when metarubricytes and more immature cells of this system are seen. This polychromasia should not be confused with a blue cast given to all RBCs in a peripheral blood smear from overstaining.

Erythrocyte or mature RBC. The erythrocyte, or mature red blood cell (normocyte), normally averages 7 to 8 micrometers in diameter. The cell is biconcave, and this causes a difference in the intensity of cytoplasmic staining. There is no nucleus. The cytoplasm at the periphery of the cell is moderately buff-pink (orange), while the center zone is less intensely stained.

Exercises (035):

Match each of the types of RBCs in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. Large cell, 12 to 15 micrometers in diameter, with large, centrally located nucleus and granule-free cytoplasm.

___ 2. Has one or two nucleoli, finely reticulated nuclear chromatin, and no evidence of hemoglobin formation in cytoplasm.

___ 3. Contains a round, slightly eccentric or off-centered nucleus, cytoplasmic granules are not present, and there is no evidence of hemoglobin formation in the cytoplasm.

___ 4. Chromatin is coarse and clumped with distinct parachromatin, no nucleoli are present, and the cytoplasm shows intense basophilia.

___ 5. Chromatin material is coarse and granular with distinct parachromatin, sometimes described as a spoke-wheel pattern.

___ 6. The cell has a small, sometimes round or sometimes bizarre, bilobed, clover leaf nucleus which stains intensely basophilic (blue-black).

___ 7. The cytoplasm is polychromatophilic due to the first appearance of the hemoglobin.

___ 8. The cytoplasm is orthochromatic (buff-pink), and the nucleus is basophilic and homogeneously intense.

___ 9. The immediate precursor of the mature erythrocyte.

___10. Since the reticulum is not demonstrable in Wright stained preparations, supravital staining distinguishes these from those with diffused basophilia and basophilic stippling, both demonstrable with Wright stain.

___11. Caused by residual basophilic substance from precursors.

Column B

a. Erythrocyte.
b. Reticulocyte.
c. Rubricyte.
d. Rubriblast.
e. Metarubricyte.
f. Diffuse basophilia.
g. Prorubricyte.

___12. The cytoplasm at the periphery of the cell is moderately buff-pink, while the center zone is less intensely stained.

036. Match a list of morphological variations in red blood cells and their associated disease processes with appropriate descriptive statements.

Variations in Erythrocytes. The formation of red cells (erythropoiesis) is regulated by the intake of substances to build the cells, the storage of these substances, and their use. In normal erythropoiesis, the cytoplasm and the nuclei of cells grow at a synchronized rate. Individual differences in physiology and physical structure of the erythrocyte account for minor morphological changes. In certain diseases, these morphological changes may vary greatly.

Pernicious anemia. Probably the most striking of these variations is that observed in megaloblastic anemias, of which *pernicious anemia* is one example. Pernicious anemia is a disease in which vitamin B_{12} is not absorbed. The erythrocytes do not mature normally and are larger than normal in people with this deficiency. The most notable characterictic of this abnormal maturation is a difference in the rates of maturation of the cytoplasm and the nucleus. The nucleus develops more slowly than the cytoplasm, so that in the more mature nucleated forms a finely reticulated chromatin net is seen in the nucleus. This is in contrast to the coarser clumped chromatin observed in more mature nucleated forms in normal maturation. Such development is termed "asynchronism." The mature cell is large (about 10 micrometers) and is termed a *megalocyte* (macrocyte). The young cells of this series are named by adding the suffix "pernicious anemia type"; for example, metarubricyte, pernicious anemia type.

Other factors may produce morphological variations in red blood cells. For example, genetically controlled variations in the hemoglobin may result in changes in morphology to sickle shapes, elliptocytes, or target cells. Iron deficiencies may cause striking changes wherein erythrocytes contain only a small thin rim of hemoglobin; for example, hypochromia. In another variant, the erythrocytes are small and spherical rather than bioconcave disks. Let's discuss some of the more common morphological variations that occur in erythrocytes. A brief description accompanies each variant. These variations are readily seen when blood smears are stained with Wright stain. Refer to foldout 1 as you study each of the following abnormal forms of the erythrocyte.

Crenated erythrocytes. Crenated erythrocytes have serrated or prickly outlines resulting from shrinkage of the cells. This may occur when blood films dry too slowly and the surrounding plasma becomes hypertonic. This deformity is artificial and has no pathological significance, except when crenated cells are found in spinal fluid as discussed in the preceding section concerned with performing cell counts on cerebrospinal fluid.

Hypochromic erythrocytes. Hypochromic erythrocytes have an increased central pallor as a result of decreased hemoglobin content. Extreme hypochromia, in which only a narrow rim of hemoglobin remains at the cell periphery, is called oligochromasia.

Polychromatophilia. The term "polychromatophilia" (polychromasia) refers to nonnucleated erythrocytes which have a blue tinge instead of the normal buff-pink or orange color. The cytoplasm of these cells is not completely mature, resulting in abnormal persistence of the basophilic (blue) cytoplasm of the earlier nucleated stages. Polychromatophilia denotes erythrocyte immaturity.

Spherocytes are spherical in shape, have a diameter smaller than normal, have greater fragility than normal erythrocytes, and are without central pallor. These cells are found in hemolytic diseases and are particularly characteristic of congenital spherocytosis, a Mendelian hereditary disorder.

Anisocytosis. The term "anisocytosis" is used to describe a situation in which we find erythrocytes of several different sizes in the same blood specimen. It is a frequent finding in many types of anemia; it is particularly pronounced in pernicious anemia, where megalocytes and normocytes are found together. Cells smaller than 6 micrometers in diameter are spoken of as microcytes, while those larger than 9 micrometers are called macrocytes.

Poikilocytes. Poikilocytes are irregularly shaped erythrocytes. They may be pear shaped, elliptical, or comma shaped, or may occur in various other forms. Poikilocytosis results from abnormal erythropoiesis or from mechanical factors such as a change in oxygen tension, osmotic influences, or other forces. Crenated cells are not usually considered true poikilocytes. Hereditary anomalies may predispose the formation of poikilocytes when conditions for their transformation are optimal.

Sickle cells. Sickle cells are abnormal red cells which assume a half-moon or sickle-shaped appearance under conditions of reduced oxygen tension. The phenomenon, due to the presence of hemoglobin "S," occurs primarily in Negroes. Sickling is demonstrated by mixing a drop of blood with a reducing agent such as a *fresh* solution of sodium metabisulfite. It is possible to demonstrate sickling in individuals who are not homozygous and hence possess the sickle cell trait but do not show clinical symptoms. Consequently, the observation of this phenomenon does not necessarily mean that the patient has the anemia. The sickle cell trait occurs in 10 percent of all Negroes in the United States, but only about 1 percent have sickle cell anemia. Hemoglobin electrophoresis, which was discussed briefly in CDC 90411, is considered a more definitive procedure. In clinical cases of sickle cell anemia, abnormal erythrocytes may be observed on the routine differential smear.

Target cells. Target cells are red cells which have more deeply stained centers and borders separated by a pale ring, giving them a targetlike appearance. Large numbers of these cells are characteristic of hemoglobinopathies but may also occur in kidney or liver disease and are normally found in patients who have had a splenectomy.

Howell-Jolly bodies. Howell-Jolly bodies are nuclear remnants found in the erythrocytes in various anemias. They are round, dark violet in color, and about 1 micrometer in diameter. Generally, only one Howell-Jolly body will be found in a red cell. However, two or more may sometimes be present.

Cabot rings. Cabot rings (ring bodies) are blue, threadlike rings found in the red cells of patients with severe anemias. They are remnants of the nuclear membrane and appear as a ring, or "figure eight" structure. Usually only one such structure will be found in any one red cell.

Basophilic stippling. Basophilic stippling (punctate basophilia) is the occurrence of round, small, deeply basophilic granules of varying size in the cytoplasm of the red cell. They are ribonuclear proteins which normally disappear as the cell matures. Basophilic stippling can be demonstrated by standard staining techniques in contrast to reticulocyte filaments which require a special stain. Stippling occurs in anemias, where it denotes cell immaturity. In heavy metal poisoning (lead, zinc, silver, mercury, bismuth), basophilic stippling is apparently the result of abnormal reactions of cytoplasmic structures in the RBC precursors.

Heinz-Ehrlich bodies. Heinz-Ehrlich bodies are small inclusions found primarily in hemolytic anemias induced by toxins. They are round, refractile bodies inside the erythrocyte and visible only in unfixed smears. It is thought that they are denatured hemoglobin and indicate erythrocyte injury.

Siderocytes. Siderocytes are erythrocytes containing iron deposits. These deposits indicate an incomplete reduction of iron from the ferric to the ferrous state that is normally found in hemoglobin. Prussian blue stain must be used to readily demonstrate these cells.

Acanthocytes. Acanthocytes are spherocytic erythrocytes with long spiny projections. This is a congenital abnormality associated with low serum concentration of low density (beta) lipoproteins. It is inherited as a recessive genetic trait.

Burr cells. Burr cells are triangular or crescent-shaped RBCs with one or more spiny projections on the periphery. These are seen in small numbers in uremia, carcinoma of the stomach, and peptic ulcer.

Pappenheimer bodies. Pappenheimer bodies are deposits of iron in the red blood cell. With Wright stain, they appear as purple granules. They are often confused with siderocytes, but the siderocyte granules will be visible only with Prussian-blue stain for iron.

Exercises (036):

Match each of the abnormal variations of erythrocytes or associated disease conditions in column B with the appropriate descriptive statement in column A by placing the letter of the column B item beside the letter of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. The nucleus develops more slowly than the cytoplasm; the mature cell is large and is termed a megalocyte.
___ 2. This may occur when blood films dry too slowly and the surrounding plasma becomes hypertonic.
___ 3. This abnormality is artificial and has no pathological significance except when found in spinal fluid.
___ 4. These cells show an increased central pallor as a result of decreased hemoglobin content.
___ 5. Nonnucleated erythrocytes which have a blue tinge instead of the normal buff-pink or orange color.
___ 6. Denotes erythrocyte immaturity.
___ 7. Are spherical in shape, have a diameter smaller than normal, have greater fragility than normal RBCs, and are without central pallor.
___ 8. Erythrocytes of several different sizes in the same blood specimen.
___ 9. They may be pear shaped, elliptical, or comma shaped.
___ 10. These cells assume a half-moon appearance under reduced oxygen tension.
___ 11. Hemoglobin electrophoresis is the test of choice for confirmatory studies of these cells.
___ 12. These cells have deeply stained centers and borders separated by a pale ring.
___ 13. Nuclear remnants found in the RBCs in various anemias; are round, dark violet in color, and about 1 micrometer in diameter.
___ 14. Are blue, threadlike structures found in the RBCs of patients with severe anemias. Remnants of the nuclear membrane appear as a "figure eight."
___ 15. Ribonuclear proteins which normally disappear as the cell matures.
___ 16. Round, small, deeply basophilic granules of varying size in the cytoplasm of the red cell.
___ 17. Round refractile bodies inside the RBCs; visible only in unfixed smear.
___ 18. Small inclusions found primarily in hemolytic anemias induced by toxins.
___ 19. Prussian blue stain must be used to readily demonstrate these cells containing iron deposits.
___ 20. Deposits of iron in the RBCs; with Wright stain they appear as purple granules.

Column B

a. Howell-Jolly bodies.
b. Basophilic stippling.
c. Heinz-Ehrlich bodies.
d. Cabot rings.
e. Siderocytes.
f. Pappenheimer bodies.
g. Acanthocytes.
h. Burr cells.
i. Pernicious anemia.
j. Anisocytosis.
k. Polychromatophilia.
l. Crenated erythrocytes.
m. Hypochromic erythrocytes.
n. Spherocytes.
o. Target cells.
p. Poikilocytes.
q. Sickle cells.

___21. Cells are crescent-shaped RBCs with one or more spiny projections on the periphery; seen in small numbers in uremia, carcinoma of the stomach, and peptic ulcer.

___22. Spherocytic RBCs with long, spiny projections; associated with serum concentration of low density lipoproteins.

3-2. Abnormalities in Erythrocyte Production and Destruction

The number of red blood cells in an adult remains fairly constant. The total number or volume of erythrocytes can be measured by special tests. Knowledge of the total red blood cell volume and total plasma volume may help the physician to determine the amount of replacement blood or intravenous fluids to be given to a patient.

The red blood count based on the total erythrocyte volume may be subject to a slight, even hourly, variation. In the normal individual, this range of variation remains essentially the same throughout adult life. The rate of destruction of aged and work-out cells is approximately the same as the rate of production. If the production-destruction ratio is not in equilibrium, either more or fewer red cells than normal will result. If the number of red cells is increased, the condition is known as polycythemia; if it is decreased, lowering the hemoglobin concentration, the condition is known as anemia.

037. Identify the three types of polycythemia in terms of their causes and physiological conditions.

Polycythemia. There are three types of polycythemia. The most common is known as relative polycythemia. This term describes increase in blood cell concentration resulting from plasma loss, as in burns, and dehydration from excessive vomiting, diarrhea, sweating, or stress.

The second most common form of polycythemia is known as secondary polycythemia. It is frequently a response to hypoxia (insufficient oxygen). Secondary polycythemia may result from prolonged exposure to high altitude, anoxia due to congenital heart diseases, or chronic exposure to those chemicals which inactivate the oxygen-carrying potential of hemoglobin, that is, nitrate and carbon monoxide.

The third type of polycythemia is known as polycythemia vera, a condition of unknown cause. Polycythemia vera is characterized by a widespread stimulation of the entire bone marrow, with overproduction involving granulocytes and platelets as well as erythrocytes.

Exercises (037):

1. What is relative polycythemia?

2. What are some causes of secondary polycythemia?

3. What condition causes polycythemia vera?

4. What physiological characteristic is found in polycythemia vera?

038. Point out terms, factors, conditions, and indications associated with the various types of anemias.

Anemia. Anemia can be manifested by a decrease in the quantity or an alteration in the quality of the erythrocytes. Anemia is a symptom rather than a disease; however, the classification of anemias is both difficult and complex. From your standpoint, the classification of anemias is basically concerned with size, shape, color, and inclusions of the red cells. The normal red cell is 7 to 8 micrometers in diameter. Variation in size of cells is called anisocytosis, as defined earlier in this chapter. Remember, too, that large cells are called macrocytes and the cells that are smaller than normal are called microcytes.

Normal stained erythrocytes are orange or buff-pink colored due to hemoglobin content; they are called normochromic. A cell that has only a small rim of color is said to be hypochromic. Hyperchromia does not exist, because the erythrocytes do not exceed more than 33 to 36 grams of hemoglobin per deciliter of cells.

Pathogenesis, inherited or acquired. On the basis of pathogenesis, the anemias may be classified into those caused by deficient hemopoietic maturation factors (the hemoglobin deficiency anemia), and those due to deficiency in numbers of red cells. Exclusive of iron, normal hematopoiesis depends upon factors in the diet; intrinsic factor; vitamin B_{12} and folic acid; gastric absorption, and liver storage factors. Deficiencies in any or all of these factors will result in the megaloblastic maturation typical of pernicious anemia and sprue.

We have already learned that inherited abnormalities of hemoglobin (hemoglobinopathies) can cause definite anemic conditions such as sickle cell anemia and thalassemia. Sickle cell anemia is characterized by the typical sickle-shaped cell, whereas thalassemia exhibits target cells. Acquired hemoglobin disorders may be caused by endocrine disorders, infection, poisons, or radiation.

Blood loss and hemolytic states. In hemorrhagic or hemolytic states, red cells may lose their ability to perform normal functions. Chronic, slight hemorrhage may not cause anemia, since the system frequently compensates for a small loss. However, if the loss of blood exceeds production, an iron deficiency anemia may result. This will cause the red blood cells to be smaller than normal, a condition known as microcytosis. In addition to chronic blood loss, this hypochromic, microcytic anemia may result from a poor diet, malabsorption, or poor iron utilization. With acute hemorrhage, the blood loss is so great that the body does not have ample time to compensate for the loss.

Hemolysis causes the liberation of hemoglobin and results in a shortened cellular survival. Thus, there may be an increase in the serum bilirubin, urine urobilinogen, and stercobilinogen (fecal urobilinogen). The free hemoglobin that is released affects the body's metabolism by impairment of the oxygen-carrying capacity of the red blood cells. Such an anemia is logically called hemolytic anemia. Congenital hemolytic anemia is recognized by abnormal cellular morphology (elliptocytes, spherocytes), abnormal hemoglobin ("C," "S," etc.), or both.

Acquired hemolytic states. Acquired hemolytic anemia may be caused by bacterial or parasitic infections such as malaria; chemicals or drugs such as arsenic, lead, and sulfonamides; or from extensive burns.

Conditions causing a weakening of the cellular membrane result in hemolysis. These conditions are associated with antibodies, such as the cold and warm hemolysins, and the immune substances of the ABO and Rh categories. Special tests are required for the detection of the cause of hemolysis brought on by immunologic factors. Such tests are the Coombs test, tests for cold and warm hemolysins and agglutinins, and the Ham test for paroxysmal nocturnal hemoglobinuria. Paroxysmal nocturnal hemoglobinuria is defined as rather sudden, recurring hemoglobinuria due to hemolysis during sleep.

Indications of hemolytic anemia. Rarely, the first indication of the presence of a hemolytic type of anemia is the phagocytosis of erythrocytes. Antibodies that cause hemolysis can also stimulate phagocytosis of erythrocytes.

Quite often an increased demand on the hematopoietic system due to excessive loss of blood will result in the release of immature and atypical forms of erythrocytes into the circulation. These immature forms may be an increased number of reticulocytes, basophilic stippled RBCs, or even metarubricytes. Atypical forms are those in which the rate of development of the nucleus and the cytoplasma vary from the normal.

The most common of these forms is the erythrocyte that has a homogeneous, basophilic substance diffused throughout the cytoplasm (polychromasia). Other forms result from the deposition of the nuclear material into configurations such as Cabot rings and Howell-Jolly bodies described earlier in the chapter.

Exercises (038):

1. What factor concerning RBCs, other than a decrease in quantity, may indicate anemia?
2. Define "normochromic" and "hypochromic" in terms of the amount of color in stained erythrocytes.
3. List three factors upon which normal hematopoiesis depends.
4. What is hemoglobinopathy?
5. Name two hemoglobinopathies.
6. What are some causes of acquired hemoglobin disorders?
7. In addition to chronic blood loss, producing hypochromic microcytic anemia, what are other possible causes?
8. How does free hemoglobin, released from the red cells by hemolysis, affect their oxygen-carrying capacity?
9. What term is used to describe a hemolytic anemia manifested by abnormal cellular morphology, abnormal hemoglobin, or both?
10. What are some causes of acquired hemolytic anemia?
11. How do antibodies cause hemolysis?

039. Match the types of anemias with descriptive statements concerning their cellular morphology, symptoms, and specific conditions.

Other Types of Anemias. Studies of the anemias are based on symptoms of the disease and the type of red cells produced. Blood indices and reticulocyte counts are helpful in evaluation of the different forms. Fragility tests that determine the degree of elasticity or strength of the cell membrane and studies of the cellular morphology are further aids to evaluation. PCV (packed cell volume) and hemoglobin studies are among the most useful tests to evaluate anemias. A few of the more common types of anemias are described below.

Microcytic hypochromic anemia. This is a common anemia characterized by red cells smaller than normal in size and with reduced hemoglobin content. This is an iron deficiency anemia which develops as a result of poor diet, malabsorption or poor utilization of iron, or chronic blood loss.

Hemolytic disease of the newborn (HDN). This is a hemolytic anemia occuring in the newborn. It is usually the result of incompatibility between fetal antigens and maternal antibodies. It is characterized by hyperplasia of the bone marrow and the presence in peripheral blood of large numbers of rubricytes and reticulocytes.

Acute hemolytic anemia. This condition is characterized by symptoms of rapid blood destruction. It may be the result of malaria, septicemia, bartonellosis, a variety of chemical agents, extensive burns, snake venoms, or circulating antibodies. The blood picture is usually that of a normocytic anemia with anisocytosis, a high reticulocyte count, and varying numbers of other immature erythrocytes. White blood cells and platelets may also be increased. The osmotic erythrocyte fragility test is usually normal. The bone marrow is hyperplastic.

Congenital hemolytic anemia (hereditary spherocytosis). This is a hereditary, hemolytic anemia characterized by an increased erythrocyte osmotic fragility and a variable number of small spherocytes, erythrocytes, and reticulocytes. Polychromatophilia and, rarely, metarubricytes may also be seen in the peripheral blood. Another type of congenital hemolytic anemia which is nonspherocytic results from a hereditary deficiency of glucose-6-phosphate-dehydroginase (G-6-PD) in the erythrocytes. However, it is much rarer than the congenital spherocytic anemia.

Mediterranean anemia (Cooley's anemia or thalassemia). This is a chronic, progressive, hereditary anemia commencing early in life and affecting persons of Mediterranean origin. It is characterized by the presence of thin, target-type red cells in the peripheral blood. These cells have a decreased osmotic fragility. Immature red and white cells in the peripheral blood reflect the marrow activity.

Pernicious anemia. This is a chronic macrocytic, normochromic anemia caused by a defect in the production of "intrinsic factor" by the stomach mucosa. There is megaloblastic hyperplasia of the bone marrow. The peripheral blood shows macrocytosis, poikilocytosis, polychromatophilia, granulocytopenia, and granulocytic hypersegmentation. Clinical features include achlorhydria and neurological disturbances.

Aplastic anemia. This is a severe anemia due to interference with blood formation which may follow exposure to a great variety of chemical or physical agents, such as benzene, (glue-sniffing,) arsenic, gold salts, atomic and X-radiation, and certain antibodies. Or the disease may be of unknown origin.

With few exceptions, most red cell abnormalities are observable on stained blood smears. A few, such as sickle cell anemia, abnormal sedimentation of red cells, and increased cell destruction rate, require preparations designed to measure particular characteristics of abnormal red blood cells. A few of these preparations that evaluate abnormal cells are discussed in the next section.

Exercises (039):
Match each of the given types of anemias in column B with the appropriate descriptive statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. Characterized by red cells smaller than normal in size, with reduced hemoglobin content.
___ 2. The result of incompatibility between fetal antigens and maternal antibodies.
___ 3. An iron deficiency type of anemia developing from poor diet, malabsorption, poor utilization of iron, or chromic blood loss.
___ 4. Characterized by increased bone marrow production and the presence of large numbers of rubricytes and reticulocytes in the peripheral blood.
___ 5. Symptoms of rapid blood destruction possibly resulting from malaria, septicemia, bartonellosis, a variety of chemical agents, extensive burns, snake venoms, or circulating antibodies.
___ 6. Normocytic anemia with anisocytosis, a high reticulocyte count, and varying numbers of other immature erythrocytes.
___ 7. Hereditary, hemolytic anemia characterized by increased erythrocyte osmotic fragility and varying numbers of spherocytes.

Column B

a. Pernicious anemia.
b. Congenital hemolytic anemia.
c. Hemolytic disease of the newborn (HDN).
d. Aplastic anemia.
e. Mediterranean anemia.
f. Acute hemolytic anemia.
g. Microcytic hypochromic anemia.

___ 8. Chronic progressive, hereditary anemia commencing early in life and characterized by the presence of thin target-type red cells in the peripheral blood.

___ 9. Chronic macrocytic, normochromic anemia caused by a defect in the production of "intrinsic factor" by the stomach mucosa.

___10. The peripheral blood shows macrocytosis, poikilicytosis, polychromatophilia, granulocytopenia and granulocytic hypersegmentation.

___11. May be caused by prolonged exposure to any of the following: benzene (glue sniffing), arsenic, gold salts, radioactivity, and certain antibodies.

3-3. Evaluation of Red Cell Abnormalities

Several of the techniques mentioned in this chapter which evaluate or demonstrate erythrocyte morphology are used primarily as screening procedures in lieu of technically difficult, time-consuming procedures. In this section we will briefly comment on a few of these methods.

040. Indicate whether given statements correctly reflect procedures, sources of error, and interpretation of the sodium metabisulfite and dithionite tube methods of screening for sickle cell disease.

Sickle Cell Screening Test. A diagnosis of sickle cell disease must be confirmed by electrophoresis, but a simple screening test is usually used first. The screening test is based on the principle that red cells that contain hemoglobin S will form sickle shapes when placed under lowered oxygen tension. Persons with the sickle trait (genetically heterozygous) will show a positive sickle cell preparation even though they do not have the disease. Several other abnormal hemoglobins—for example, hemoglobins I, C, Georgetown, and Bart—are also reported to sickle in this preparation. It is interesting to note that in homozygous S infants less than 4 months old RBCs sometimes will not sickle because their fetal (F) hemoglobin has not been sufficiently replaced by adult hemoglobin.

Sodium metabisulfite method. This test will show sickling if positive by mixing 1 drop of blood specimen with two drops of 2 percent sodium metabisulfite ($Na_2S_2O_5$) solution. Three-grain tablets of the metabisulfite salt, which are commercially available, are sufficient to prepare 10 ml of reagent. It is important to prepare fresh reagent on the day it is used. The preparation should stand for 30 minutes before microscopic examination. Particular attention should be paid to the edges of the preparation before reporting as negative. The test should be reported only as positive or negative for sickling, since neither the morphology nor the number of sickle cells is significant. With this method it is difficult to distinguish sickle cell trait from sickle cell anemia, even though in sickle cell anemia, the sickling reaction occurs more rapidly than in sickle cell trait. This is, however, a poor criterion on which to rely.

Dithionite tube test method. A sample of 20 μl of whole blood is added to 2.0 ml of dithionite working solution and mixed in a 12- x 75-mm test tube. After 5 minutes the tube is examined for turbidity against a lined reader, as indicated in figure 3-1. Hemoglobin S, if present, produces turbidity in the tube. If there is no hemoglobin S or non-S sickling hemoglobin present, the solution will be clear and the lines on the reader scale will be visible through the solution. Hemoglobin electrophoresis should be performed if the dithionite tube test is positive. The dithionite tube test and urea-dithionite test may be adapted to the automated method and performed on the Auto Analyzer.

Sources of error. The dithionite reagent has a limited stability, and the freshness should be checked with positive and negative controls. The size of the tubes is important. The use of 10- x 75-mm tubes may result in a false negative. Fresh blood samples are not necessary for the test, since reliable results have been obtained on specimens up to 20 days old. Unstoppered tubes containing dithionite reagent decompose when left out at room temperature. False negative results could occur if the blood sample for testing is drawn within 4 months of transfusion.

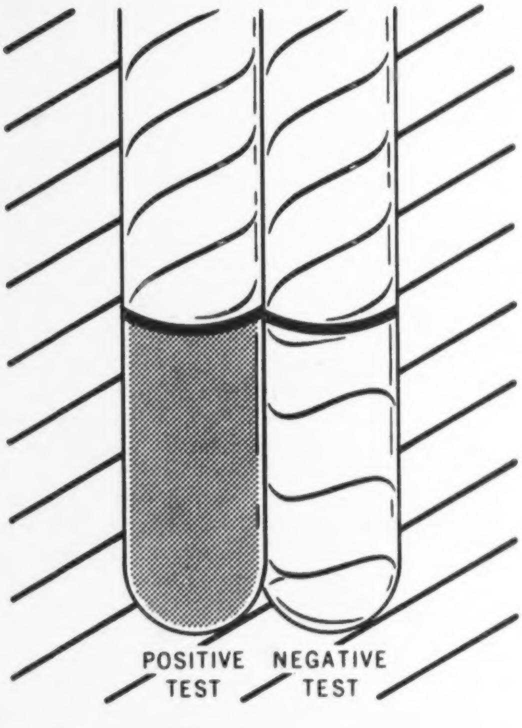


Figure 3-1. Dithionite tube test interpretations.

Interpretation. Hemoglobin S causes turbidity in the tube. Hemoglobin A is soluble in the phosphate buffer. Hemoglobin S is an inherited type of hemoglobin found in Negroes and in people from Mediterranean areas. The degree of erythrocyte sickling is dependent on the concentration of hemoglobin. SS, SC, and SD cells sickle more rapidly than AS cells. Newborns with sickle cell anemia have erythrocytes more resistant to sickling due to the present of hemoglobin F.

The dithionite test also detects other sickling types of hemoglobin. Urea causes hemoglobin S (and structural variants of hemoglobin S) to dissolve. Other hemoglobins remain turbid in the presence of urea.

This test is a rapid screening test for hemoglobin S. All positive tests should be electrophoresed for confirmation. A positive sodium dithionite tube test may also be found in hemoglobin Bart, hemoglobin C-Harlem, and the presence of certain abnormal proteins.

Exercises (040):

If one of the following statements is correct, mark it true; if it is false, correct it.

T F 1. The screening test for sickle cell disease by the sodium metabisulfite method is based on the principle that red cells containing hemoglobin S will form sickle cell shapes under lowered oxygen tension.

T F 2. Persons with the sickle cell trait (genetically heterozygous) will not show a positive sickle cell preparation.

T F 3. Homozygous "S" infants less than 4 months old have RBCs more resistant to sickling due to the presence of hemoglobin F, fetal hemoglobin.

T F 4. With the sodium metabisulfite method for sickle cell screening, it is easy to distinguish sickle cell trait from sickle cell anemia by the morphology and number of sickle cells present.

T F 5. In the dithionite tube test, 0.2 ml of blood is added to 2.0 ml of dithionite working solution.

T F 6. In the dithionite tube test, if there is no hemoglobin S or nonsickling hemoglobin present, turbidity will be noted in the tube.

T F 7. If the dithionite tube test is positive, a hemoglobin electrophoresis is unnecessary.

T F 8. The sodium metabisulfite and the urea-dithionite tests may be adapted to the automated method and performed on the Auto Analyzer.

T F 9. If 12- x 75-mm test tubes are the only tubes available for the dithionite tube test, they may be used with no possible effect on the accuracy of the test.

T F 10. Fresh blood samples are not necessary for the dithionite tube test.

T F 11. A false negative finding could result if blood is drawn within 4 months of transfusion.

T F 12. Hemoglobin S is an inherited type hemoglobin found in Negroes and in people from Mediterranean areas.

T F 13. Hemoglobin SD cells sickle more rapidly than do AS cells.

T F 14. A positive dithionite tube test result may be found in thallasemia.

041. Point out the ways in which the erythrocyte sedimentation rate is affected by three main factors, and state the significance of ESR.

Erythrocyte Sedimentation Rate (ESR). The sedimentation of blood has been studied since antiquity. The introduction of ESR into modern medicine came when it was proposed as a test for pregnancy in 1918. In clinical medicine today, the ESR is used in a general sense to detect, confirm, and/or follow the course of a disease process. Its interpretation involves judgments which only the attending physician is equipped to make. There are three main factors which affect the ESR: size and shape of erythrocytes, composition of plasma, and mechanical, or technical, factors.

Erythrocytes. A factor of great significance in determining the rate of fall of erythrocytes is the size or mass of the falling particle. The larger the particle, the faster the rate of fall. Normally, the red blood cells will remain more or less separated from each other. They are negatively charged and repel each other (zeta potential). In certain diseases, however, plasma proteins, especially fibrinogen and globulins, may be altered causing rouleaux formation. This condition results in a larger cell mass and an increased sedimentation velocity. Changes in the red blood cells' surface, resulting from agglutination, also lead to an increased red cell mass and a more rapid sedimentation. Figure 3-2 indicates the two conditions discussed that will increase the sedimentation's velocity. Macrocytes tend to settle at a faster rate than microcytes. Elevation of ESR is noted in severe macrocytosis. Erythrocytes showing alteration in their shape, such as sickle cells and spherocytes, are unable to agglutinate or form rouleaux; thus their sedimentation rate will be decreased. A markedly elevated sedimentation rate is noted in severe anemia. The erythrocyte concentration in the blood is decreased, and therefore they settle out more easily and rapidly.

Because of the complexity of factors involved in the ESR, it has become evident that correction of the ESR for only anemia (reduction in total cell volume) is of questionable validity. Therefore, it is recommended by most authorities that the ESR no longer be corrected. However, since anemia does affect the ESR, though not a linear function, it is recommended that the hematocrit be reported with each ESR. Whenever indicated, both corrected and uncorrected sedimentation rates may be reported.

Composition of plasma. The single most important factor determining the ESR is the composition of the plasma. Rouleaux and red blood cell aggregation are affected mainly be the levels of fibrinogen, alpha-1 globulin, and alpha-2 globulin. Both rouleaux and RBC aggregation will increase in proportion to the increasing levels of these three plasma proteins.

Mechanical and technical factors. There are also several purely technical aspects of performing the ESR correctly. For instance, the ESR will vary with the length and bore size of the ESR tube. This is not a consideration in most USAF laboratories since the Wintrobe tube is almost universally used. However, if microtubes are used for infants, the normal values show corresponding change. The sedimentation tube must also be perpendicular during the test. Usually this is controlled by a leveling bubble on the tube support rack. The level should be checked before each ESR is begun. The ESR will be increased if the tube is not perpendicular. The tube rack should also be placed on a sturdy table that is not subject to vibrations from centrifuges or other equipment. Such vibrations will increase the ESR.

Significance of the ESR. Normally, children have a lower ESR than adults. Adults over 60 years old will frequently show higher levels than normal adults. The ESR is a nonspecific response to tissue damage and will denote the presence of disease, but not its severity. It is often used to follow the progress of certain diseases such as tuberculosis and rheumatism.

Exercises (041):

1. How is the ESR used in modern clinical medicine?

2. In considering the erythrocyte as a factor affecting the ESR, what three circumstances increase the mass, causing greater sedimentation velocity?

3. What two plasma proteins are altered, causing rouleaux formation?

4. Why does presence of sickle cells and spherocytes cause a decreased ESR?

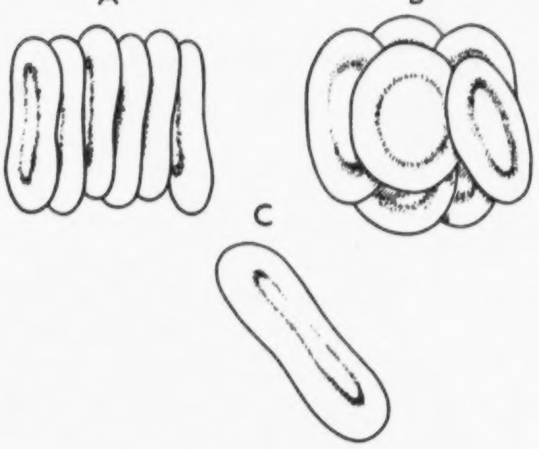


Figure 3-2. Erythrocyte conditions causing increase in sedimentation velocity.

5. Why is there a significantly increased ESR in severe anemia?

6. What three plasma protein levels are responsible for rouleaux formation and RBC aggregation?

7. If the sedimentation tube is not perpendicular during the test, what condition will result?

8. Normally, when the ESR of a child is compared with that of an adult over 60 years old, which is higher?

9. How can the ESR denote the severity of disease?

042. Specify anticoagulants used for the ESR, other physical factors affecting the ESR, and sources of error.

Anticoagulants. There are differences in ESR related to the anticoagulant. First of all, a liquid anticoagulant should *not* be used because of the dilution effect. It is obvious that a true packed cell volume cannot be determined if the blood has been diluted with a liquid anticoagulant for the Wintrobe ESR method. When this dilution factor is introduced, the values tend to be higher. The ESR is not affected by liquid EDTA (0.05 ml, 7.5 percent or 15 percent solution). Further, during blood collection, an optimal amount of blood must be mixed with the anticoagulant to prevent distortion of the RBCs. For instance, in a 5-ml vacuum tube with double oxalate, no less than 3 ml of blood must be collected. Double oxalate is the most time-honored anticoagulant used; however, technically it is probably second best to either heparin or EDTA as an anticoagulant. No appreciable RBC shrinkage is reported with either heparin or EDTA anticoagulants, but up to 5 percent lower hematocrit values have been reported using double oxalate. Regardless of the anticoagulant used, it is most important to use the same one consistently with all ESRs performed in your laboratory.

Other physical factors. Marked changes will occur in the ESR 3 hours after collection. Because of this, it is recommended that the blood specimen be used within 2 hours. The specimen should not be refrigerated or warmed. During settling of the cells, appreciable changes in the sedimentation rate will occur in temperatures outside the range of 22° to 27° C. Temperature correction charts are available to correct for slight changes in temperature alone. You should remember, however, that extremes of temperature may change certain factors; for example, plasma proteins, which also affect the ESR.

Sources of error. Some possible sources of error are as follows:

a. The blood specimen must be properly mixed with the proper anticoagulant to obtain an undiluted representative sample.

b. Delay in performing the test beyond 2 hours after the blood is drawn decreases the sedimentation rate.

c. Increase in temperature accelerates the rate.

d. The tube must be vertical. A 3° variation from the vertical accelerates the rate by 30 percent.

e. Dirty Wintrobe tubes or capillary pipettes can decrease the rate.

Exercises (042):

1. Blood was obtained in a vacutainer tube for prothrombin time (anticoagulant, 0.5 ml sodium citrate). What effect will this have on the ESR if the sample was used?

2. What percent of RBC shrinkage is noted with heparin or EDTA?

3. What is the desirable temperature range for ESR?

4. Delay in performing the test beyond 2 hours after the blood is drawn produces what effect on the ESR?

5. By what percent is the sedimentation rate accelerated when the tube is more than 3° from the vertical?

043. List the three red cell indices used in the classification and study of anemias and the determinations necessary to calculate these values; given the necessary information, calculate indices.

Red Cell Indices. Three red cell or erythrocyte indices are used in the classification and study of anemias: the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC). These indices are based on three determinations: the red blood cell count, the hemoglobin, and the hematocrit. A discussion of each of the three indices, its calculation, and the interpretation follows.

Mean corpuscular volume (MCV). MCV is the mean volume of an erythrocyte in the RBC population of the speciment. It is expressed in femtoliters, formerly called cubic microns. Lower than normal values are obtained iln microcytosis, and higher than normal in macrocytosis. Because MCV is a mean value, it is possible to have a normal MCV and yet have microcytes and macrocytes present (anisocytosis). It is obvious that microscopic observation must be made to validate the MCV value. The formula for calculating the MCV is as follows:

$$\text{MCV} = \frac{\text{hematocrit} \times 10}{\text{red cell count (in millions)}}$$

$$= \text{femtoliters (fl), formerly c}\mu\text{ (cubic microns)}$$

Example:

Hematocrit = 45 percent
Red count = 5,000,000 per cm^3
Normal values: 82 to 92 fl

The MCV indicates whether the erythrocytes will appear normocytic, microcytic, or macrocytic. The red blood cells will be microcytic if the MCV is less than 82 fl. If the MCV is greater than 92 fl, the red blood cells will be macrocytic. The red blood cells will be normocytic if the MCV is within the normal range.

Mean corpuscular hemoglobin (MCH). The MCH indicates the average weight of hemoglobin in the red blood cell.

$$\text{MCH} = \frac{\text{hemoglobin (g per dl)} \times 10}{\text{red cell count (in millions)}}$$

Example:

Hemoglobin = 15 g per dl
Red count = 5,000,000 per cm^3

$$\frac{15 \times 10}{5} = \text{30 picograms (pg), formerly micromicrograms } (\mu\mu\text{g})$$

Normal values: 27 to 31 pg

The MCH indicates the amount of hemoglobin in the red cell and should always correlate with the MCV and MCHC. The MCH will be elevated in macrocytic anemias and in some cases of spherocytosis in which hyperchromia may be indicated.

Mean corpuscular hemoglobin concentration (MCHC). The MCHC is an expression of the average concentration of hemoglobin in the red blood cells. It gives the ratio of hemoglobin to the volume of the red blood cells, and is expressed in grams per deciliter of red cells. Since a normal RBC contains the maximum concentration of hemoglobin, this value can only be reported as normal or below normal. The formula for finding the MCHC is:

$$\text{MCHC} = \frac{\text{hemoglobin (g/dl)} \times 100}{\text{hematocrit}}$$

$$= \text{g/dl (grams per deciliter, formerly percent)}$$

Example: Given a hemoglobin determination of 15 g/dl and a hematocrit determination of 45 percent, find the MCHC.

$$\text{MCHC} = \frac{15 = 100}{45} = 33 \text{ g/dl}$$

Normal values range from 32 to 36 g/dl.

The MCHC indicates whether the red blood cells will be normochromic, hypochromic, or hyperchromic. Normal values for MCV and MCH in newborns and infants are higher. MCHC normals are the same for infants and adults. Although automatic counters which give all parameters of blood cell indices are available in many facilities, knowledge of the derivation of the formulas aids in understanding the results.

Exercises (043):

1. List three values which comprise the red cell indices.

2. What three values must be determined for calculations of RBC indices?

3. Calculate the MCV if the hematocrit is 42 percent and the red count is 6,000,000 per cm^3.

4. Would the MCV in exercise 3 be considered microcytic, normocytic, or macrocytic?

5. Calculate the MCH if the hemoglobin is 12 g/dl and the RBC count is 4,000,000 per cm^3.

6. Which one of the indices gives the ratio of hemoglobin to the volume of red blood cells?

7. Calculate the MCHC if the hemoglobin is 14 g/dl and the hematocrit is 45.

044. Describe the erythrocyte osmotic fragility test by citing the principle upon which it is based, the three stages of hemolysis, factors that affect the results of the test, and a procedure that increases the test's sensitivity.

Erythrocyte Osmotic Fragility Test. The erythrocyte osmotic fragility test is used to help diagnose different types of anemia in which the physical properties of the red cell are altered.

Red blood cells are excellent indicators of osmotic pressure. The degree of distortion or eventual lysis which occurs to an RBC in a fluid is directly related to the osmotic pressure between the cell and the surrounding fluid.

An erythrocyte goes through several stages in the process of lysis. In the first stage, the normal biconcave disc crenates; then the cell becomes spheroid along with crenation. Finally, the crenated spherocyte loses its crenation and hemolyzes, and a ghost cell (representing stroma without hemoglobin) remains. Since a spherocyte has progressed to a late stage of this system, very slight osmotic changes will cause it to hemolyze. You will recall that in congenital spherocytic anemia, the erythrocyte osmotic fragility is increased. It is also increased in hemolytic disease of the newborn, chemical poisoning, and burn cases. In sickle cell anemia and several other conditions, the RBC fragility test is decreased.

The test itself is quite simple to perform. You are familiar with the term "isotonic" saline or 0.85 percent sodium chloride solution. In isotonic saline, the osmotic pressures are balanced between the red blood cell and saline so that there is no cellular distortion or hemolysis; whereas, in distilled water the osmotic pressures are so imbalanced between the two that hemolysis of the cells occurs immediately upon exposure. The osmotic fragility test is based upon these phenomena. The test consists of a series of progressively lower salt concentrations from 0.85 percent to a 0 percent solution (distilled water). When erythrocytes are placed in these solutions, the RBCs will lyse at a certain concentration below 0.85 percent (initial hemolysis), and will lyse completely (complete hemolysis) between the initial hemolysis concentration and distilled water. The salt concentrations must be very exact for clear-cut reactions. The test report should state the concentration at which both initial and complete hemolysis occurred. Normally, initial hemolysis occurs between the saline concentrations of 0.42 to 0.46 percent and is complete between 0.30 to 0.34 percent concentrations.

In one modification of the fragility test, the amount of hemolysis is determined by measuring the amount of hemoglobin released. The percentage of hemoglobin in each tube is reported, equating the "complete hemolysis" tube to 100 percent released hemoglobin.

Another modification which greatly increases the sensitivity of the RBC fragility test involves incubating the blood specimen at 37° C for 24 hours before running the test. For this modification, the blood must be collected aseptically in a sterile container with glass beads. The specimen is rotated before testing so that the blood is defibrinated by the glass beads.

Exercises (044):

1. What physical characteristic of a fluid affects the distortion of lysis of RBCs in that fluid?

2. In the process of lysis what happens to the cell in the first stage?

3. What condition occurs in the second and final stages?

4. In what conditions will the osmotic fragility be increased?

5. Why is a red blood cell *not* lysed in isotonic (0.85 percent) saline?

6. What may be done to increase the sensitivity of the RBC osmotic fragility test?

CHAPTER 4

Leukocyte and Thrombocyte Maturation

EACH OF THE FIVE types of white blood cells originate from primitive cells which have similar morphological characteristics. As primitive cells change to "blasts" and more mature types, their nuclear and cytoplasmic characteristics change. These changes are distinctive enough to differentiate the types of cells from each other. Thrombocytes (platelets) also originate from an early-type cell that undergoes changes and finally fragments into the numerous platelets found in blood.

4-1. Normal Maturation Sequence

A study of leukocyte and thrombocyte maturation necessarily involves studying the various stages that the cells pass through prior to assuming the characteristics of mature cells. These stages of development are generally referred to as the normal maturation sequence.

045. List changes observed in general maturation of blood cells and granules observed in granulocyte maturation, and name the cell stages in normal maturation order of the granulocytic series.

Development of Blood Cells. Developing blood cells follow a set series of events as the cell approaches maturity. Mature cells are, as a rule, smaller than immature cells. The nuclear chromatin becomes more clumped and compact as the cell matures. Also, the staining reaction of the cytoplasm changes as a cell matures. The younger cells are generally more basophilic than mature cells. Cells in the granulocytic, agranulocytic, and megakaryocytic series all demonstrate the above characteristics. These and other special differences form the basis for our discussion of the maturation of the three classes of cells mentioned above.

Granulocytic Series. Specific cytoplasmic granules develop in cells of the granulocytic series as the cells mature. Nonspecific granules (azurophilic) will develop initially after the blast stage and be found in early stages of the granulocytic series. As you read the description of cells in the granulocytic series, refer to foldout 2 in the back of the volume.

At birth, the granulocytic leukocytes originate in the bone marrow. A secondary potential is maintained throughout life in the reticuloendothelial system, which includes the spleen and liver. As the granulocyte matures, azurophilic, nonspecific cytoplasmic granules give way to more specific granules. Normally, only band and segmented granulocytes are observed in peripheral blood. The other cells are normally seen only in bone marrow preparations. The stages in the normal maturation of the granulocytes are:

a. Myeloblast.
b. Promyelocyte.
c. Myelocyte (neutrophilic, eosinophilic, and basophilic).
d. Metamyelocyte (neutrophilic, eosinophilic, and basophilic).
e. Band cell (neutrophilic, eosinophilic, and basophilic).
f. Segmented cell (neutrophilic, eosinophilic, and basophilic).

Exercises (045):

1. List four general trends observed in the maturation of all blood cells.

2. What granules are normally observed first in the maturation of granulocytes?

3. Where do the granulocytic leukocytes originate and what secondary potential system exists throughout life?

4. Name the cell stages in the normal maturation order of the granulocytic series.

046. Identify the distinctive characteristics of the myeloblast, promyelocyte, and myelocyte/neutrophilic myelocyte in terms of the size, nucleus, and the cytoplasm content.

Granulocytic Cells. Formation of the granulocytic series of leukocytes normally takes place in the bone marrow. During the development stage from the myeloblast to the promyelocyte, nonspecific granules are formed. These granules vary in staining from blue to reddish-purple. The formation of nonspecific granules will cease as the cell matures, and the cell will begin to form specific granules.

The development of the granulocytic cells shows distinctive characteristics in the various stages as described in the following paragraph.

Myeloblast. The distinguishing characteristics of myeloblasts are as follows:

Size. Myeloblasts range from 10 to 20 µm (micrometers) in size.

Cytoplasm. The amount of cytoplasm is small in relationship to the rest of the cell. The cytoplasm is very smooth in texture, is usually nongranular, and forms a thin rim around the nucleus.

Nucleus. The nucleus is round or oval. It occupies about four-fifths of the cell, and has a delicate, interlaced chromatin pattern. The nucleus stains reddish-purple and contains two to five nucleoli.

Promyelocyte. Promyelocytes have the following characteristics:

Size. They range from 14 to 20 µm in size.

Cytoplasm. The nuclear-cytoplasmic (N:C) ratio is 5:1 as opposed to 7:1 for the myeloblast. Cytoplasm stains pale blue. A few large, nonspecific azurophilic granules are present. More mature promyelocytes may show a few specific granules (neutrophilic, basophilic, or eosinophilic).

Nucleus. The nucleus of promyelocytes is oval or round and occupies one-half or more of the cell. It contains two to three nucleoli which are not as distinct as in the myeloblast. The chromatin is slightly clumped, appearing more coarse and less evenly stained. A cell ceases to be a promyelocyte and becomes a myelocyte when specific, definitive granules are present and the nucleus becomes slightly indented.

Myelocyte. Myelocytes have the following distinguishing characteristics:

Size. They range from 12 to 18 µm in size.

Cytoplasm. The cytoplasm contains a few to moderate number of nonspecific granules. Specific granules begin to appear at this stage, and are easily distinguished as being neutrophilic, eosinophilic, or basophilic by the specific granulation.

Nucleus. The nuclei of the myelocytes are oval or round. The chromatin pattern becomes coarser, and nucleoli are either not visible or absent. Nuclei may be centrally located or eccentric. This is the last stage capable of cell division.

Neutrophilic Myelocyte. The first sign of neutrophilic differentiation is a small relatively light area of ill-defined, pink granules which develop in the cytoplasm among the nonspecific azurophilic granules. The azurophilic granules become less prominent and disappear as the myelocyte ages. The chromatin appears more clumped in the myelocyte than in the promyelocyte. Neutrophilic myelocytes are usually smaller than promyelocytes and have a relatively larger cytoplasm. The N:C ratio is approximately 2:1.

Exercises (046):

Match each type of granulocytic cell in column B with the appropriate descriptive statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

_____ 1. There is a small amount of cytoplasm in relationship to the rest of the cell.
_____ 2. Nucleus has delicate interlaced chromatin pattern.
_____ 3. N:C ratio is 5:1.
_____ 4. N:C ratio is 7:1.
_____ 5. Two to three nucleoli, not too distinct, with chromatin slightly clumped and less evenly stained.
_____ 6. Specific granules may be seen in the mature cells of this stage.
_____ 7. The last stage capable of cell division.
_____ 8. Oval or round and nucleus occupies one-half or more of the cell.
_____ 9. Definitive granules are present and the nucleus becomes slightly indented.
_____ 10. First sign is indicated by a small area of ill-defined pink granules which develop in the cytoplasm among the azurophilic granules.
_____ 11. Chromatin appears more clumped than in promyelocyte.
_____ 12. Usually smaller than promyelocyte and has a relatively larger cytoplasm.
_____ 13. As this cell ages, the azurophilic granules become less prominent and disappear.

Column B

a. Promyelocyte.
b. Neutrophilic myelocyte.
c. Myeloblast.
d. Myelocyte.

047. Identify the distinctive characteristics of the metamyelocyte, band granulocyte, and segmented granulocyte.

Metamyelocyte (Juvenile). Metamyelocytes have the following distinguishing characteristics:

Size. They range from 10 to 18 µm.

Cytoplasm. The amount of cytoplasm is moderate to abundant. Nonspecific granules are not seen, but there is a full amount of specific granules. Nuclear-cytoplasmic (N:C) ratio is about 1.5:1.

Neutrophilic metamyelocyte: The granules are pinker and numerous.

Eosinophilic metamyelocyte: Granules are brighter orange-red and more numerous.

Basophilic metamyelocyte: The dark purple granules are numerous.

Nucleus. The nucleus of the metamyelocyte is kidney shaped or indented, with a heavy nuclear membrane. The chromatin pattern is coarser and has no visible nucleoli. The chromatin stains deep purple, much darker than that of younger cells.

Additional characteristics. Neutrophilic metamyelocytes are slightly smaller than myelocytes and have a relatively smaller nucleus and less defined chromatin net. Neutrophilic metamyelocytes are not seen in the peripheral blood of normal individuals, but are often found in acute conditions in which there is a marked increase in myelocytic proliferation.

Band Granulocyte (Band). The band granulocyte has the following distinguishing characteristics:

Size. These cells range from 10 to 15 μm in size.

Cytoplasm. Their cytoplasm is similar to that of the metamyelocyte.

Nucleus. The nucleus is sausage or band shaped. The chromatin pattern is coarse. It may be constricted at one or more points, with a sufficient amount of chromatin visible in the constriction. The nucleolus is not visible.

Additional characteristics. In the band form, the nuclear indentation is more indented than the kidney-shaped nucleus of the metamyelocytes but does not have filaments typical of the segmented neutrophil. You should recall at this point that there are normally 3 to 5 percent of band forms in peripheral blood. Neutrophilic bands are slightly smaller than metamyelocytes. The specific cytoplasmic granules of band neutrophils are small and evenly distributed, and stain various shades from lilac to pink with Wright stain.

Segmented Granulocyte. The segmented granulocyte may be distinguished by the following characteristics:

Size. Segmented granulocytes are 10 to 15 μm in size.

Cytoplasm. The N:C ratio is 1:3; color is light pink or blue. Specific granules are:

a. Neutrophilic. These are fine granules, pink or lilac in color.

b. Eosinophilic. These granules are large and brick red.

c. Basophilic. These granules are large and blue-black.

Nucleus. The nucleus of a segmented granulocyte appears clumped with a coarse chromatin pattern. It stains deep purple-blue with scant parachromatin. Two or more lobes of nuclear chromatin are connected by thin filaments.

Additional characteristics. Mature neutrophils are approximately twice the size of erythrocytes. These cells differ from neutrophilic bands in that the nucleus has two or more definite lobes separated by very thin filaments rather than an indentation. The cytoplasm in an ideal Wright-stained preparation is buff or pink, and the small, numerous, and evenly distributed neutrophilic granules have a lilac color.

The transition between the various stages of neutrophilic cells is gradual. Differentiation of cells is made almost exclusively from the nuclear configuration. Borderline cells are difficult to distinguish. Cells should be placed in a more mature category when there is doubt; however, in the distinction between bands and segmented neutrophils, the assumption that a filament must be present even if one cannot be seen should *not* be made. Such cells would be classified as bands. If a question arises as to whether a cell is a metamyelocyte or a band, it should be counted as a band cell.

Eosinophils. Eosinophils are normally found in small numbers in bone marrow and peripheral blood smears.

Basophils. Basophils are so few in peripheral blood and bone marrow that there is no clinical advantage in differentiating them into two separate development stages. There are fewer granules present than found in eosinophils. These granules have dissolved in the process of staining and washing and are water soluble.

Exercises (047):

Match each type of granulocytic cell in column B with the correct statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

_____ 1. The granules are pinker and numerous; cytoplasm is abundant; size is 10 to 18 μm; the nucleus is kidney shaped; nuclear membrane is heavy and has coarser chromatin pattern; nucleus stains darker than that of younger cells.

_____ 2. The granules are brighter orange-red and more numerous; cytoplasm is abundant; size is 10 to 18 μm; the nucleus is kidney shaped; nuclear membrane is heavy and has coarser chromatin patterns; nucleus stains darker than that of younger cells.

_____ 3. Granules are dark purple and numerous; cytoplasm is abundant; size is 10 to 18 μm; the nucleus is kidney shaped; nuclear membrane is heavy and has coarser chromatin pattern; nucleus stains darker than younger cells.

Column B

a. Neutrophilic segmented cell.
b. Neutrophilic band cell.
c. Basophilic metamyelocyte.
d. Segmented granulocyte.
e. Band granulocyte.
f. Neutrophilic metamyelocyte.
g. Eosinophilic segmented cell.
h. Eosinophilic metamyelocyte.
i. Eosinophilic band.
j. Basophilic segmented cell.

_____ 4. Are slightly smaller than myelocytes and have relatively smaller nucleus and less defined chromatin net.

_____ 5. Not normally seen in the peripheral blood but often found in acute conditions in which there is myelocytic proliferation.

_____ 6. Cytoplasm is abundant; nucleus is sausage or band shaped. Sufficient amount of chromatin is visible in the constriction, if any; granules are pinker and more numerous; size is 10 to 15 μm.

_____ 7. Nuclear indentation is more indented than kidney shaped, but does not have filaments typical of segmented neutrophils.

_____ 8. Cytoplasm is abundant; nucleus is sausage or band shaped; sufficient amount of chromatin is visible in the constriction, if any; granules are large, bright orange-red, and numerous.

_____ 9. Nucleus is clumped, with coarse chromatin pattern; staining is purple-blue with scant parachromatin; two or more lobes of nuclear chromatin are connected by thin filaments; granules are fine and pink or lilac.

_____ 10. Nucleus is clumped, with coarse chromatin pattern; staining is purple-blue with scant parachromatin; two or more lobes of nuclear chromatin are connected by thin filaments; granules are large and blue-black.

_____ 11. Cells should be reported in this manner when they are borderline between metamyelocyte and band cells.

_____ 12. Granules dissolve in the process of staining and washing and are water soluble.

048. Indicate whether given statements correctly reflect the characteristics and features pertaining to the cells of the megakaryocyte series.

Megakaryocyte Series. Microscopic evaluation of the megakaryocytes in bone marrow may be of importance in coagulation problems involving thrombocytes (platelets), since thrombocytes are produced by the megakaryocytes.

The cells of the megakaryocytic series grow larger as they mature until there is cytoplasmic fragmentation to form the thrombocytes seen in peripheral blood. Azurophilic granulation begins to appear in the second stage of development and continues until it almost obscures the nuclear lobes. The nucleus develops from a discrete round or oval shape to multiple ill-defined lobes. The stages in the normal maturation of the megakaryocytic series are:

- Megakaryoblast.
- Promegakaryocyte.
- Megakaryocyte.

Megakaryoblast. These cells range from 20 to 30 microns in diameter and are irregular in shape. There are two to six small nucleoli in the nucleus. The cytoplasm is scant, irregularly basophilic, and agranular.

Promegakaryocyte. These cells are generally larger than the preceding cells, ranging up to 50 micrometers in diameter. They may have an indented or double nucleus. A few azurophilic granules may be present in the cytoplasm around the nucleus. The cytoplasm may show some polychromasia and initial platelet formation.

Megakaryocytes. These are the largest blood cells, ranging from 40 to 100 microns in diameter. They have ill-defined multilobed nuclei with clumped chromatin. The cytoplasm has irregularly dispersed, fine azurophilic granules and may show some fragmentation of platelets. Megakaryocytes do not appear in the peripheral blood. In bone marrow preparations, there should be little difficulty in recognizing cells as being of this series because of their enormous size.

Exercises (048):

Identify each true statement, and explain why the others are false.

T F 1. Evaluation of the megakaryocytes in bone marrow may be of importance in coagulation problems involving thrombocytes.

T F 2. Thrombocytes are produced by the plasmocytes.

T F 3. The promegakaryocytes are the direct precursors of thrombocytes.

T F 4. The megakaryoblasts range from 20 to 30 micrometers in diameter and are irregular in shape; two to six nucleoli are present.

T F 5. Promegakaryocytes are smaller than megakaryoblasts, ranging up to 15 micrometers in diameter.

T F 6. In the promegakaryocyte, few azurophilic granules may be present in the cytoplasm around the nucleus, and the cell may show an indented nucleus.

T F 7. Megakaryocytes are the largest blood cells, ranging from 40 to 100 micrometers in diameter. They have ill-defined, multiobed nuclei with clumped chromatin.

T F 8. Megakaryocytes do not appear in the peripheral blood and are easily recognized in bone marrow preparations because of their enormous size.

049. Cite the origin of lymphocytes, and identify the distinguishing morphological characteristics of a lymphoblast, a prolymphocyte, and a lymphocyte.

Agranulocytic Series. The agranulocytic series is composed of leukocytes without specific granulation. This series includes lymphocytes and monocytes. Lymphoblasts, the lymphocyte precursors, originate primarily in the lymphatic system. Few are found in normal bone marrow, even though the marrow is involved in lymphopoiesis. Monoblasts are probably derived from hemocytoblasts, the precursors of myeloblasts, and from hemohistioblasts, which are the most primitive cells oriented to hemopoiesis. The monoblasts mature to form promonocytes and then monocytes. Refer to foldout 2 as you study the cell types described.

The stages in the development of the lymphocytic series are:

- Lymphoblast.
- Prolymphocyte.
- Lymphocyte.

Lymphoblast. These cells are similar to other blast cells. The nucleus is round or oval, staining light red-purple with Wright stain. The nuclear chromatin is finer than that of the lymphocyte but not as delicate as in the myeloblast. There is a moderate amount of light blue parachromatin. One or two nucleoli are present. The cytoplasm is moderately basophilic and nongranular, forming a thin rim around the nucleus.

Prolymphocyte. Some authors do not differentiate this cell from the lymphoblast. When these cells are differentiated, it is more by comparison with lymphoblasts and lymphocytes than by any unique, morphological characteristics. The nuclear chromatin is described as more coarse than in the lymphoblast, being slightly clumped. One nucleolus is usually present. There is more abundant, light blue to moderately basophilic cytoplasm. In addition, there may be a few azurophilic granules. Generally, the prolymphocyte is smaller than the lymphoblast.

Lymphocyte. The lymphocyte is more distinctive morphologically than its precursor. The cell is described as approximately 6 to 20 micrometers in diameter, or from about the size of a normal erythrocyte to more than twice the size of a red cell. The cytoplasm may vary in quantity from scant to moderate, depending upon the thickness of the smear and the size of the cell. The cytoplasm is normally clear and homogeneous, and may be described as light blue, sky blue, lightly basophilic, or moderately basophilic. The cytoplasm may normally contain a few reddish-violet or azurophilic granules which are peroxidase negative. A clear perinuclear zone is often observed in the cytoplasm. The nucleus of a normal lymphocyte may be round, oval, or slightly indented. It contains clumped chromatin, which appears in Wright-stained preparations as very dark staining bluish-purple aggregates in the nucleus separated by lighter staining, indistinct areas of parachromatin. This description of the normal lymphocyte indicates the wide biologic variations which must be thoroughly appreciated before attempting to differentiate the normal from the atypical lymphocyte. Later in this chapter, we will discuss atypical lymphocytes.

Exercises (049):

1. Primarily, where do lymphocytes originate in the body?

2. What cell is described by the following characteristics?
   Cell size: 6–20 micrometers in diameter; about twice the size of an RBC.
   Quantity of cytoplasm: From scant to moderate.
   Cytoplasm histochemistry: Light to moderate basophilic, staining light to moderate blue.
   Nucleus: Round, oval, or slightly indented.
   Granules: Reddish-violet or azurophilic, peroxidase negative.
   Chromatin: Clumped, dark staining bluish-purple aggregates separated by indistinct areas of parachromatin.

3. When the lymphoblast is compared with the prolymphocyte, which cell is generally smaller?

4. How does the nuclear chromatin of the lymphoblast compare with that of the lymphocyte and the myeloblast?

050. Identify the most distinctive morphological features of the monoblast, promonocyte, and monocyte.

Monocyte. The stages in the development of a monocyte are:

- Monoblast.
- Promonocyte.
- Monocyte.

Monoblast. This cell is extremely difficult to differentiate from the myeloblast. The nucleus is round or oval and appears more lightly stained than in the myeloblast. The nuclear chromatin is fine and delicate with abundant, sharply defined, pale pink or blue parachromatin. One or two nucleoli are present. There is a moderate amount of basophilic or grey-tinged cytoplasm. There are no granules in the cytoplasm.

Promonocyte. Some authorities do not differentiate the promonocyte from the monoblast. Descriptions sometimes differentiate the two cells by the presence of perhaps one nucleolus and very fine, lilac staining granules in the cytoplasm of the promonocyte. The granules are so small that they are called azurophilic dust.

Monocyte. Mature monocytes are usually larger than other leukocytes in peripheral blood (15 to 25 micrometers). Unstained monocytes exhibit slow, amoeboid movement and may be seen on the stained slide with single or multiple pseudopods.

The nucleus of the monocyte is usually folded, but it may be round, kidney shaped, or deeply indented. One of the most distinctive features of the monocyte is the very fine, diffuse chromatin strands with abundant parachromatin in the nucleus. This diffuse, very light staining nucleus differentiates the monocyte from the lymphocyte and metamyelocyte. This delicate chromatin pattern is in contrast to the lymphocyte and metamyelocyte chromatin, which is clumped. Nucleoli are absent. The cytoplasm of a monocyte is opaque, gray-blue without the clear perinuclear zone described for most lymphocytes. It has been described as "foamy" or having a ground glass appearance. There is a large amount of cytoplasm in relation to the nucleus, which is also in contrast to the lymphocyte. The nonspecific, fine, azurophilic granules of the monocyte are dustlike and lilac staining. A few large, unevenly distributed azurophilic granules may be seen in overstained smears. If overstained, the monocyte may be confused with metamyelocytes.

Monocytes may be difficult to differentiate from other cells, particularly if the stain is not good. In poorly stained blood smears, the delicate nuclear morphology will appear less distinct and the coarse granules will confuse the picture. When other differential features are absent, the brainlike convolutions of the nucleus and the dull gray-blue color of the cytoplasm are usually sufficient to classify the cell as a monocyte.

The classification of normal blood cells into specific categories is sometimes difficult. Sometimes it is not possible to do so. While normal blood cells, as a rule, follow the patterns we have discussed, they don't always do so. If the technician becomes thoroughly familiar with the normal characteristics of a particular type of cell, he will be more aware of atypical cells when he sees them and will be better prepared to evaluate the abnormal cells that are considered in the following section.

Exercises (050):

1. How does the cytoplasm of the monoblast compare with that of the promonocyte?

2. How does the nucleus of the monoblast compare with that of the promonocyte?

3. What characteristic is exhibited by the unstained mature monocyte? Stained slide?

4. How does the nucleus of the monocyte appear?

5. Name one of the most distinctive morphological features of the monocyte.

6. How does the cytoplasm of the monocyte compare with that of the lymphocyte?

7. If overstained, the monocyte may be confused with what type cell?

8. When other differential features are absent, what may be used to sufficiently classify the cell as a monocyte?

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4-2. Leukocyte Abnormalities

The vital properties of the leukocytic response to inflammation enable us to study leukocytes, since they may use the pathway of circulation to reach the site where they function. Experimental and clinical data indicate that distinctive patterns of leukocytic response can be anticipated in different types of diseases. Thus, as an aid to diagnosis, observation can be made from leukocytes in the peripheral blood and the distribution of various types of leukocytes.

051. Specify the cause and appearance of compressed or distorted cells, and name two terms used to describe leukocytes resulting from mechanical pressure.

Cell Distortion Artifacts. Blood cells may show distortion artifacts. Distorted cells occur as a result of compression or crushing. Compression is due to the pressure of cells upon each other, and crushing is caused by mechanical pressure which ruptures cells when the smear is made. Compressed cells appear smaller than normal with darker staining cytoplasm. Monocytes and lymphocytes are easily crushed. The monocyte, when crushed, may exhibit a U-shaped nuclear remnant. The lymphocyte will develop "spindle forms." Abnormal crushed cells are the "smudge" or "basket cells," which are immature or fragile leukocytes. These smudge cells are found predominantly in diseases with an acute shift toward immature forms, for example, leukemias.

Exercises (051):

1. What conditions cause compression and crushing of cells?

2. How does the size and staining of the cytoplasm of compressed cells compare with other normal cells?

3. How resistant to crushing are monocytes and lymphocytes?

4. Name two terms used to describe leukocyte remnants in a differential resulting from mechanical pressure.

052. Identify the terms used to designate increases and decreases in the various types of white cells.

Terminology of WBC Increases and Decreases. The terminology used to designate increases or decreases in the various types of white cells is shown in table 4-1. A leukocytosis is present if the total circulating leukocyte count is above 10,000/mm^3 and a leukopenia is present if the leukocyte count is less than 5,000/mm^3.

Leukopenia. A leukopenia due to a decrease in the number of neutrophils is specially referred to as "neutropenia." A leukopenia also includes the lymphocytes and other white blood cells. A leukipenia, or count of 4,000 per mm^3 due to the reduction in neutrophils, could show 50 percent lymphocytes.

Leukocytosis. An understanding of leukocytosis, leukopenia, and the differential leukocyte count is essential before studying the leukemias, which might be confused with diseases accompanied by leukocytosis. In Leukemias there is an increase in the numbers of the abnormal and primitive cells in the peripheral blood as well as in the bone marrow.

Polycythemia vera. In polycythemia vera, a benign disease which may be confused with leukemia, there is an absolute increase in all stages of the leukocytes, blood platelets, and erythrocytes. Histochemical studies of cells from cases of polycythemia vera indicate that the cells are probably normal. Sometimes in polycythemia vera, granulocytic leukemia develops.

Neutrophilic leukocytosis. Infections with pyogenic bacteria cause striking examples of neutrophilic leukocytosis. Localization of the infection with pus under tension may cause a severe leukocytosis. Then again, the infection may be so severe or overwhelming that leukocyte production is depressed rather than stimulated. Marked leukocyte responses occur in infancy and childhood, while in older individuals the changes are not as marked. Generally, acute infections cause leukocytosis, while chronic infections may fail to stimulate production of leukocytes and are associated with neutropenia. In infections due to viruses, there may be an initial neutrophilic leukocytosis. A relative or absolute lymphocytosis with neutropenia is usually present.

Neutrophilic leukocytosis is almost always due to a hyperplasia of certain cells in the bone marrow. The blood picture reflects the degree of production of these cells in response to the body need. A shift to the left in the differential count means that more immature cells are being liberated from the bone marrow due to an unusual demand; for example, in acute bacterial infections. A shift to the right means more mature cells with some neutrophils with hyperlobation and more degenerative forms are present. In the presence of a neutropenia, it is indicative of reduced marrow production of neutrophils.

Leukemoid states. In leukemoid states, the blood picture may be that of a true leukemia. However, when a disease is accompanied by anemia, thrombocytopenia, splenomegaly, hepatomegaly, or lymph node enlargement, it may be difficult to distinguish the leukemoid reaction from leukemia. A leukemoid blood picture may involve either the granulocytic or

TABLE 4-1
TERMINOLOGY APPLICABLE TO INCREASES OR DECREASES IN THE PRINCIPAL TYPE OF WHITE CELLS

Type of cell	*Increase termed*	*Decrease termed*
Neutrophil	Neutrophilia	Neutropenia
Eosinophil	Eosinophilia	Eosinopenia
Basophil	Basophilia	Basopenia
Lymphocyte	Lymphocytosis	Lymphocytopenia
Monocyte	Monocytosis	Monocytopenia

agranulocytic series. A great increase in the leukocytes without extreme immaturity suggests a leukemoid reaction. However, a shift to the left so extreme as to show promyelocytes, and a rare "blast" form, may occur in leukemoid reactions. Also, achronic granulocytic leukemia may show few "blast" forms in the blood, and a bone marrow aspiration may be necessary to differentiate the two. Even the bone marrow study may not be diagnostic. Granulocytic leukemoid reactions most commonly occur in whoopcough, chicken pox, and infectious monoucleosis, and when bone marrow is replaced by metastatic tumor.

Exercises (052):
Match each of the following terms in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

_____ 1. Total circulating leukocyte count is 17,000/mm³.
_____ 2. Total circulating leukocyte count is 2,000/mm³.
_____ 3. Term used to indicate a significant decrease in the number of neutrophils.
_____ 4. An increase in the numbers of abnormal and primitive cells in the peripheral blood as well as in the bone marrow.
_____ 5. A benign disease which may be confused with leukemia; there is an absolute increase in all stages of the leukocytes, blood platelets, and erythrocytes.
_____ 6. Histochemical studies of cells appear to be normal, and in some cases, granulocytic leukemia may develop in this disease.
_____ 7. Striking examples may be caused by infections with pyogenic bacteria.
_____ 8. Chronic infections may fail to stimulate the production of leukocytes and are associated with this condition.
_____ 9. Is almost always due to a hyperplasia of certain cells in the bone marrow.
_____ 10. Term used to indicate a significant increase in neutrophils.
_____ 11. Term used to indicate a significant increase in eosinophils.
_____ 12. Differential count in which more immature cells are being liberated from the bone marrow due to an increased demand.
_____ 13. Term used to indicate a significant increase in lymphocytes.
_____ 14. Term used to indicate a significant decrease in lymphocytes.
_____ 15. Differential count in which more mature cells with some neutrophils with hyperlobation and more degenerative forms are present.
_____ 16. A condition suggested when there is a great increase in the leukocytes without extreme immaturity.

Column B

a. Neutrophilia.
b. Lymphocytopenia.
c. Neutropenia.
d. Lymphocytosis.
e. Eosinophilia.
f. Leukopenia.
g. Leukocytosis.
h. Leukemoid/ reaction.
i. Polycythemia vera.
j. Neutrophilic/ leukocytosis.
k. Leukemia.
l. Eosinopenia.
m. Shift to the left.
n. Shift to the right.

053. Cite distinctive features of infectious mononucleosis and infectious lymphocytosis in terms of lymphocyte morphology, tests, and associated conditions.

Infectious Mononucleosis. Infectious mononucleosis is a self-limiting disease thought to be caused by a virus. The disease affects children or young adults, and has an incubation period of 10 to 14 days. The onset is characterized by fever, sore throat, and enlarged cervical lymph nodes. There may be involvement of the whole body, especially the lymphatic tissues of the reticuloendothelial system, with symptoms and signs varying with the organs and tissues involved.

Diagnosis of I.M. Diagnosis of infectious mononucleosis is aided by microscopic examination of blood, the heterophil antibody test, and the monospot test. The specificity of the monospot test is excellent and as good as that of the standard differential absorption test. Occasionally, a bone marrow biopsy may be necessary to rule out leukemia. Leukocytosis may be present in the early stages before enlargement of the lymph glands. However, the total leukocyte count may be normal or low. There is an increase of both lymphocytes and monocytes. These two types of cells usually average 60 percent of the total count. A differential count containing less than 40 percent agranulocytic cells usually does not indicate progressed infectious mononucleosis.

Lymphocyte morphology. Atypical lymphocytes are characteristic of mononucleosis. These atypical lymphocytes or virocytes have been divided into three groups by Downey and McKinlay. This classification is less important today from a clinical viewpoint than it was a few years ago.

The classic Downey cells of infectious mononucleosis are probably the best examples of cells reported as atypical lymphocytes. This is not to say that they are the only ones. Other viral infections generate their full share of atypical cells, and very prominent among these are atypical lymphocytes. Downey's original description of the atypical lymphs of infectious mononucleosis is of historical interest, but the classification by type is not applicable, since there is no correlation of the types with the severity of the disease. Atypical lymphocytes, per se, are not pathognomonic of infectious mononucleosis. An atypical lymphocyte may be large or small. It may have vacuoles in the cytoplasm, in which case the cytoplasm has been described as "foamy" in appearance; or it may have scant or abundant cytoplasm that is deeply basophilic, staining deep blue; or, it may have both basophilic and vacuolated cytoplasm. If a lymphocyte contains definite nucleoli (lighter staining, punched-out areas in the nucleus), it is always atypical, whether it contains basophilic, vacuolated cytoplasm or not. Those lymphocytes containing nucleoli may be misinterpreted as leukemia.

The distinguishing features of the great majority of atypical lymphocytes are vacuolated cytoplasm, deeply basophilic cytoplasm, and nucleoli in the nucleus. Any one of these three morphological characteristics or any combination of these is indicative of an atypical lymphocyte, and the percentage should be reported. If there is an absolute lymphocytosis, close observation of the individual cells should be made for atypical features.

The morphologic diagnosis is of value, but the final diagnosis may depend upon serological tests, such as the monospot test or differential absorption test. These tests will be discussed in a later volume of this course. After the clinical condition subsides, the atypical cells may be seen for many months. The bone marrow picture of infectious mononucleosis is not constant in appearance and can be differentiated from leukemia.

Infectious Lymphocytosis. Infectious lymphocytosis is a contagious disease of young children, believed to be caused by a virus. Clinical manifestations are mild or may even be absent. The leukocytosis may be as high as 50,000 per mm^3, and sometimes over 100,000 per mm^3. There is both an absolute and relative increase of normal, small, mature lymphocytes. The lymphocytosis may last 3 to 5 weeks, or slightly longer. The bone marrow is not remarkable in the disease, for there is a lymphocytosis, but there may be a granulocytopenia. The monospot test is negative.

Exercises (053):

1. What tissues of the reticuloendothelial system may be especially involved in infectious mononucleosis?

2. In addition to microscopic examination of blood, what specific test may be used in the diagnosis of infectious mononucleosis?

3. How does the classification by type of atypical lymphocytes correlate with the severity of the disease?

4. What are the distinguishing features of atypical lymphocytes?

5. Which of the morphological characteristics in exercise 4 is considered more specific for classifying an atypical lymphocyte?

6. Final diagnosis of infectious mononucleosis is dependent upon what type of tests?

7. What type of lymphocytes are usually increased in infectious lymphocytosis?

8. What is the result of the monospot test in infectious lymphocytosis?

054. Define leukemia, and point out typical blood picture, method of differentiation, and diagnosis of the three common types of leukemia.

Leukemia. Leukemia may be defined as a neoplastic disease characterized by the proliferation of hematopoietic cells in the blood-forming organs and in the peripheral blood. The diagnosis should be made by a pathologist or hematologist. This is an extremely serious disease, and the treatment involves potent toxic drugs. The duration of the disease depends upon the type of leukemia and varies from a few days to as long as 20 years. The more common types of leukemia are discussed below.

Chronic myelogenous leukemia. Chronic myelogenous leukemia is characterized classically by a very high white count consisting of cells of the granulocytic series in various stages of maturation, usually with many mature forms. There is associated anemia. The platelets may be either decreased or marked by increase, giving rise to thrombotic episodes in some cases. This type of leukemia in many cases is difficult to differentiate from a leukemoid reaction even with a bone marrow aspiration. Chromosome and chromosomal studies may be quite helpful. Myelogenous leukemia is associated with chromosomal abnormality on the 21st chromosome. This is called the *Philadelphia chromosome.* The peroxidase stain is used to help differentiate leukemias. The stain indicates the presence of peroxidases normally present in granulocytic cells and to a lesser degree in monocytes. Cells in the lymphocytic series and basophils yield negative staining. The leukocyte alkaline phosphatase test is low in granulocytic leukemias and is frequently used to differentiate leukemoid reactions from leukemia.

Chronic lymphocytic leukemia. Chronic lymphocytic leukemia is a disease that usually affects patients who are older than 50. It is associated with the best prognosis among the leukemias. Some patients live as long as 20 years with the disease. Patients may be asymptomatic and yet have a 100,000 white cell count. In these circumstances, many physicians withhold treatment. The blood picture shows large numbers of mature lymphocytes, and the bone marrow, spleen, and lymph nodes are markedly affected. There are associated anemia, leukopenia, and thrombocytopenia.

Acute leukemias. The acute leukemias usually have a rapid course and are characterized by numerous blast cells in the peripheral blood. The types of acute leukemias are in many cases differentiated with difficulty. It is important, if at all possible, to differentiate myelogenous and lymphocytic varieties, because the latter are more amenable to remission with present drug therapy than the former. In acute myelogenous leukemia, thin, eosinophilic, rod-shaped structures are seen in the cytoplasm of an occasional cell. These are called Auer rods (bodies). These leukemias have associated profound anemia and thrombocytopenia. Other types of leukemia are rare and include monocytic, megakaryocytic, plasmocytic, and others.

Exercises (054):

1. Define leukemia.

2. What general white count is noted in chronic myelogenous leukemia and what type of cells are noted?

3. What test is frequently used to differentiate a leukemoid reaction from myelogenous leukemia?

4. With what chromosome abnormality is myelogenous leukemia associated?

5. As compared with the granulocytic cells, to what degree is the peroxidase stain positive for cells of the lymphocytic series and basophils?

6. What type of leukocytes are shown in the blood picture of chronic lymphocytic leukemia?

7. What are two characteristics of acute leukemia in terms of duration and blood picture?

8. Which of the acute leukemias is more amenable to remission?

9. What are Auer rods or bodies?

055. Describe the test for lupus erythematosus by citing plasma abnormality, blood morphology, distinction between tart cell and true L.E. cell, and reporting.

Lupus Erythematosus. As a final consideration in this chapter, we will briefly discuss the demonstration of L.E. cells in patients with lupus erythematosus. An L.E. cell is shown in foldout 2. This disease is characterized by the presence of an abnormal plasma protein, which results in degeneration of nuclear material, which then becomes phagocytized by other cells. This is demonstrated *in vitro* in the clinical laboratory by techniques with which you should already be familiar.

Free masses of lysed nuclear material, with or without polymorphonuclear leukocytes clustered about them (rosette formation), are suggestive of the L.E. phenomenon. Observing "rosettes" should encourage the technician to repeat the examination and search further for the true L.E. cells. A positive report should not be made without conclusive identification of this cell. A true L.E. cell is usually a neutrophilic granulocyte, but occasionally a monocyte or phagocyte, which contains a large spherical body in its cytoplasm. The nucleus of this neutrophil is pushed to one side of the cell and may appear to wrap itself around the ingested material. The inclusion within the neutrophil is a homogenous mass and has no chromatin pattern. This feature distinguishes the true L.E. cell from the "tart" cell. The tart cell, which may be confused with the L.E. cell, is usually a monocyte which has ingested another cell or the nucleus of another cell. In such case, the ingested material will often resemble a lymphocyte nucleus or phagocytized material with a recognizable chromatin pattern and nuclear membrane. Another form of ingested material found in a tart cell is an intensely stained body called a pyknotic nucleus. The significance of these cells is uncertain. However, their presence in an L.E. preparation does not indicate a positive test for systemic lupus erythematosus.

Exercises (055):

1. What condition is found in plasma of patients with lupus erythematosus?

2. What blood morphology is suggestive of the L.E. phenomenon?

3. Describe the difference between a true L.E. cell and a tart cell.

4. When pyknotic nucleus is observed as ingested material, how is the L.E. test reported?

CHAPTER 5

Blood Coagulation Studies

THE LABORATORY performs a variety of tests to assist the physician in his investigation of blood coagulation problems. Several of these tests measure the overall coagulation process, while others measure specific steps or products of this process. In most instances, prolonged bleeding is due to a deficiency of one factor or another. However, in some instances it may be due to therapeutic anticoagulants that are intentionally administered to interfere with the coagulation mechanism. In a few rare instances, prolonged bleeding is due to a natural or antigenically stimulated increase in circulating anticoagulants produced in the body.

As you progress through this chapter you will become familiar with the important aspects of the blood coagulation mechanism and the various tests used to follow bleeding disorders or anticoagulation therapy.

5-1. Principles of Coagulation

Coagulation is a complex process, and many of the participating reactions are not completely understood. Despite this fact, rational and effective therapy is available for most of the inherited and acquired disorders of the blood clotting mechanism. This is due largely to the availability of simple laboratory tests which are capable of differentiating between the many abnormal bleeding states. Modifications of these tests in recent years have clarified many of the problems previously encountered in diagnosis so that more specific therapy can be administered.

056. Define hemostasis, and point out organs, tissues, and stages of each of the three mechanisms which control the process.

Hemostasis. Before proceeding, it will be necessary to define a few basic terms. Coagulation is included in the broader concept of hemostasis. Hemostasis literally means stopping the hemorrhage of blood. Hemostasis is a complex process in which several factors work together or in sequence to stop the flow of blood from an injured blood vessel. The three main mechanisms involved in this process are: the vascular mechanism, the intrinsic thromboplastin mechanism, and the extrinsic thromboplastin mechanism.

Vascular mechanism. The vascular mechanism includes the veins, the arteries, and the capillaries themselves. The effectiveness of the vessels depends on thickness, structure, contractibility, and retractibility of their walls. The vascular component depends on blood vessels that are normal in terms of function and structure. Its physiologic importance is probably of greater significance than the mechanisms that control the coagulation of blood.

Intrinsic thromboplastin mechanism. The intrinsic thromboplastin mechanism involves coagulation of the blood itself. This is a highly complicated process that requires several stages for completion. These stages are the generation of thromboplastin, the generation of thrombin, and the production of fibrin. Each of these stages will be discussed thoroughly below. Even though the mechanism may be defined as intrinsic, it is almost impossible to avoid contaminating it with tissue juice.

Extrinsic thromboplastin mechanism. The extrinsic thromboplastin mechanism involves the surrounding tissues, such as skin, muscle, and subcutaneous tissues. The effectiveness of these tissues in slowing the flow of blood depends on their thickness, weight, tightness, and ability to contract (rubberbandlike). This mechanism also provides the body with tissue thromboplastin.

Exercises (056):

1. Define hemostasis.

2. What organs are included in the vascular mechanism, and how do they function in the hemostatic process?

3. What tissues does the intrinsic thromboplastin mechanism involve, and what three given stages are required for completion of coagulation by this mechanism?

4. What organs and tissues does the extrinsic thromboplastin mechanism involve, and by what means is the flow of blood stopped?

057. Indicate whether given statements correctly reflect the basic process of coagulation.

Coagulation. Blood coagulation is the formation of a clot from liquid blood. Abnormal plasma proteins, platelet disorders, or mechanical damage to a blood vessel may produce bleeding. In the event of a bleeding vessel, clotting is initiated by the aggregation of platelets. The platelets then congeal to form hemostatic plugs at the site of the injury. The congealing (viscous metamorphosis) process is stimulated by contact with collagen or by the formation of thrombin. Hemostasis, however, cannot be achieved without the simultaneous formation of fibrin. Therefore, as the platelets begin to aggregate and congeal, they disintegrate, releasing their contents, which contain the prothrombin activating component platelet factor 3. This factor has been abbreviated PF3; it does not have a Roman numeral assigned to it by the nomenclature committee at present.

Concurrent with the viscous metamorphosis and aggregation of platelets, other plasma factors are activated and fibrin polymerization is achieved. Arrest of bleeding is attained only when a firm fibrin network seals the blood vessel wound with enough strength to withstand the impact of intravascular pressure.

Exercises (057):
Identify each true statement, and explain why the others are false.

T F 1. Blood coagulation is the formation of a clot from the plasma.

T F 2. Bleeding may be produced by abnormal plasma proteins, platelet disorders, or mechanical damage to blood vessels.

T F 3. In the event of a bleeding vessel, clotting is initiated by the aggregation of thromboplastin.

T F 4. Hemostasis cannot be achieved without the simultaneous formation of fibrin.

T F 5. As platelets begin to aggregate and congeal, they disintegrate, releasing their contents, which contain plasma thromboplastin component (PTC) and fibrin.

058. Match a list of the coagulation factors with statements describing their properties, sites of production, and functions.

Coagulation Factors. Before proceeding into the details of the clotting mechanism, let's review the coagulation factors and consider some of their properties. In table 5-1, we have in the first column the Roman numerals which have been assigned to the factors by the International Committee for Nomenclature of Blood Clotting Factors. The common synonyms for these factors are listed in column No. 2. The site of production and a brief statement concerning their function are also given.

Factor I. Factor I, commonly called fibrinogen, is the precursor of fibrin. Factor I is produced in the liver but does not require vitamin K for its production, and it will not be present in serum. This is the one coagulation protein which can be measured by chemical means. The normal circulating level is 200–400 mg/dl. Decreases of fibrinogen are seen in an inherited disorder called afibrinogenemia. In this condition, levels are less than 50 mg percent and frequently are not measurable. Another inherited disorder is the production of an altered fibrinogen molecule, referred to as dysfibrinogenemia. Bleeding disorders do not always accompany dysfibrinogenemia. In fact, in one instance an increased tendency toward clotting is actually associated with dysfibrinogenemia. In severe liver disease we see deficiencies of fibrinogen, but in an acquired disorder, we are more apt to see fibrinogen decreases when there is a rapid *in vivo* usage of the protein.

Factor II. Factor II is commonly called prothrombin. This factor is also produced in the liver. It requires vitamin K, however, for its production. Prothrombin is the immediate precursor of thrombin. The serum will contain only a small amount of prothrombin, since it will be used up during the coagulation cycle. Hypoprothrombinemia is an inherited deficiency of prothrombin. Acquired deficiencies of prothrombin occur in liver disease, with vitamin K deficiency, and when an antivitamin K drug such as coumadin is taken.

Factor III. Factor III, commonly known as thromboplastin, is not present in the circulating blood. It is widely distributed in the body tissues. In the laboratory we use the brain and lung tissues as a source of thromboplastin for tests such as the prothrombin time.

Factor IV. Factor IV is calcium and this, of course, is present in the circulating blood. In fact, a bleeding disorder is never associated with calcium levels at

TABLE 5-1
COAGULATION FACTORS

*FACTOR	COMMON SYNONYMS	PRODUCTION	VIT K REQ'D	FUNCTION
I	Fibrinogen	Liver	No	Precursor of fibrin.
II	Prothrombin	Liver	Yes	Precursor of thrombin.
III	Thromboplastin	All tissues	No	---
IV	Calcium			Required for several reactions.
V	Labile factor Ac-globulin Proaccelerin	Liver	No	Required to form prothrombin activator.
VII	Stable factor Proconvertin	Liver	Yes	Required when tissue thromboplastin is used to form prothrombin activator.
VIII	Antihemophilic factor (AHF) Antihemophilic globulin (AHG)	RE cells	No	Required to form prothrombin activator.
IX	Plasma thromboplastin component (PTC) Christmas factor	Liver	Yes	Required to form prothrombin activator.
X	Stuart factor Prower factor	Liver	Yes	Required to form prothrombin activator.
XI	Plasma thromboplastin antecedent (PTA)	?	No	
XII	Hageman factor Contact factor	?	No	Not associated with a bleeding disorder. Initiates clotting in glass tubes.
XIII	Fibrin stabilizing factor (FSF) Plasma transglutaminase	?	No	Produces cross-linking of fibrin strands with a transglutaminase reaction.
---	Platelet factor (PF3)			Required to form prothrombin activator.

*International nomenclature.

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which life is sustained. Calcium is required for many of the coagulation reactions, and severe deficiencies may interfere with tests such as the coagulation time and the prothrombin consumption time.

Factor V. Factor V (also called labile factor, Ac or accelerator globulin, and proaccelerin) is a protein produced in the liver that does not require vitamin K for production. As the name "labile factor" denotes, factor V is lost when plasma is stored. Factor V is also very sensitive to thrombin and, if not used in the formation of the prothrombin activator, will be destroyed whenever thrombin is present. The inherited disorder associated with factor V is referred to as factor V deficiency. Acquired deficiency of factor V occurs with liver disease and also when there is a rapid utilization *in vivo* as occurs in disseminated intravascular clotting.

Factor VI. Now, factor VI does exist. Originally it was thought to be another form or an increased activity of factor V. To avoid confusion, this number has been left blank.

Factor VII. Factor VII, also called stable factor or proconvertin, is produced in the liver and requires vitamin K for its production. This protein is unique, since it is required for the formation of prothrombin activator only if tissue thromboplastin enters into the reaction. For tests such as the coagulation time, where the prothrombin activator is formed from the precursors present in the circulation, there is no need for factor VII. There is an inherited disorder known as factor VII deficiency, and acquired deficiencies of this factor are associated with liver disease, with vitamin K deficiency, and with the use of antivitamin K drugs such as coumadin.

Factor VIII. Factor VIII, more commonly known as antihemophilic factor (AHF) or antihemophilic globulin (AHG) is produced by the reticuloendothelial cells, and recently mention has been made of the role of the spleen as a site of the production of this factor. Vitamin K is not required for its production. Factor VIII plays a role in the formation of the prothrombin activator. The inherited deficiency of factor VIII is referred to as hemophilia A, or classical hemophilia. As characteristic of the hemophilias, it is a recessive sex-linked inherited disorder. Another inherited disorder in which there is a decrease in factor VIII activity is von Willebrand's disease. In this disease there is an autosomal dominant inheritance pattern. The degree of deficiency of factor VIII activity is also considerably less than that present in classical hemophilia.

Factor IX. Factor IX, plasma thromboplastin component (PTC), also known as the Christmas factor, is produced in the liver and requires vitamin K for its production. The name "Christmas factor" is derived from the surname of the first patient diagnosed by the Oxford group. Factor IX is required for the formation of prothrombin activator. The inherited deficiency of factor IX is referred to as hemophilia B, or Christmas disease. Here again there is a sex-linked, recessive inheritance pattern. Acquired deficiency of factor IX occurs with liver disease and vitamin K deficiency.

Factor X. Factor X, also known as Stuart factor or Prower factor, again from the surnames of patients, is produced in the liver. Factor X, which requires vitamin K for its production, plays a role in the formation of prothrombin activator. The inherited deficiency of the factor is called Stuart's disease or factor X deficiency. Acquired deficiency occurs in liver disease, vitamin K deficiency, or use of an antivitamin K drug such as coumadin.

At this point, let us just review. Vitamin K deficiency will result in deficiencies of factor II (prothrombin), factor VII (stable factor), factor IX (PTC), and factor X (Stuart factor). Similarly, these factors will all be reduced when coumadin, an antivitamin K drug, is taken. The degree of deficiency will depend upon the dose of coumadin and the length of time over which it is administered.

Factor XI. This factor, known as plasma thromboplastin antecedent (abbreviated as PTA), plays a role in the initiation of the coagulation cycle, leading to the formation of the prothrombin activator. The site of production of factor XI is not known, but deficiency on an inherited basis is occasionally, though not frequently, reported. This deficiency state has been given the misnomer of hemophilia C. Congenital factor XI deficiency is transmitted as an autosomal dominant trait. Other studies suggest that the gene for factor XI is incompletely recessive and the deficiency may occur as a major deficiency (homozygous) and minor deficiency (heterozygous). The inherited state is commonly referred to as factor XI deficiency.

Factor XII. Factor XII, Hageman factor (abbreviated HF), is a protein which can be activated by contact with a glass surface. In laboratory tests it plays a role in the formation of the prothrombin activator. The site of production of factor XII is not known. The inherited deficiency has been called Hageman trait or factor XII deficiency. The deficiencies of factor XII, it should be noted, are not associated with a bleeding disorder. The importance of identifying this abnormality is to explain abnormal laboratory tests without labeling the individual a bleeder.

Factor XIII. Factor XIII, called fibrin stabilizing factor (abbreviated FSF), has also been called a plasma transglutaminase more recently by it discoverers. Factor XIII is a protein which produces cross-linking of the fibrin strands to form a stable clot. The site of its production is not known. The inherited deficiency is called factor XIII deficiency. For the formation of prothrombin activator, a lipid or lipoprotein coming from the platelets is also required. This factor has been called platelet factor 3.

Exercises (058):

Match each of the coagulation factors in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

____ 1. The precursor of fibrin.

____ 2. A coagulation protein that can be measured chemically; normal values, 200–400 mg/dl.

____ 3. Requires vitamin K for its production.

____ 4. The immediate precursor of thrombin.

____ 5. Acquired deficiencies of this factor occur in liver disease with vitamin K deficiency and when an antivitamin K drug such as coumadin is taken.

____ 6. Is not present in the circulating blood and is widely distributed in the body tissues.

____ 7. Brain and lung tissues are used as a source of this substance in tests such as the prothrombin time.

____ 8. Is required for coagulation to occur; severe deficiencies may interfere with tests such as the coagulation time and prothrombin consumption time.

____ 9. Called labile factor; a protein produced in the liver that does not require vitamin K for production.

____ 10. Called stable factor; requires vitamin K for its production.

____ 11. This protein is required for the formation of prothrombin activator only if tissue thromboplastin enters into the reaction.

____ 12. A deficiency of this factor with an autosomal dominant inheritance pattern is known as von Willebrand's disease.

____ 13. The inherited deficiency of this factor is known as hemophilia A, a classical hemophilia.

____ 14. Known as the Christmas factor; produced in the liver and requires vitamin K for its production.

____ 15. Inherited deficiency referred to as hemophilia B.

____ 16. Plasma thromboplastin component (PTC).

____ 17. Inherited deficiency called Stuart's disease.

____ 18. Reduced when coumadin or other antivitamin K drug is taken.

____ 19. Plasma thromboplastin antecedent (PTA); deficiency state is given the misnomer hemophilia C.

____ 20. Hageman factor, a protein that can be activated by contact with a glass surface.

Column B

a. Factor XIII, fibrin stabilizing factor (FSF).
b. Factor X, Stuart factor.
c. Factor VII, stable factor.
d. Factor III, thromboplastin.
e. Factor I, fibrinogen.
f. Factor XII, Hageman factor.
g. Factor IX, plasma thromboplastin component (PTC).
h. Factor V, labile factor.
i. Factor II, prothrombin.
j. Factor XI, plasma thromboplastin antecedent (PTA).
k. Factor VIII, antihemophilic factor (AHF).
l. Factor IV, calcium.

____ 21. A protein which produces cross-linking of the fibrin strands to form a stable clot.

____ 22. Called platelet factor 3 (PF3) and fibrin stabilizing factor (FSF).

059. Point out the process of blood coagulation in terms of four interacting components, three stages of coagulation, and interactions involved.

Clotting Process. The clotting process theory of blood coagulation was proposed by Morowitz in a monograph published in 1905. The theory, still regarded as valid, proposed that four components interact in the presence of calcium to form a clot as follows:

$$\text{Prothrombin (Factor II)} \xrightarrow[\text{+ calcium}]{\text{Thromboplastin}} \text{Thrombin}$$

$$\text{Fibrinogen (Factor I)} \xrightarrow{\text{Thrombin}} \text{Fibrin Clot}$$

According to this working hypothesis, blood coagulation can be divided into three stages: (1) the formation of plasma thromboplastin, (2) the conversion of prothrombin to thrombin, and (3) the formation of an insoluble fibrin clot through the interaction of fibrinogen and thrombin. Further, this scheme is presumed to be applicable in either of two systems (intrinsic and extrinsic) that bring about thrombin generation. Both the intrinsic and the extrinsic systems have been interpreted in terms of an enzymic cascading mechanism. (Note fig. 5-1.)

Stage 1—The formation of plasma thromboplastin. In the *intrinsic system*, factor XII is activated *in vivo*, possibly by vascular injury and contact with collagen, resulting in XIIa. Activation of factor XII, *in vitro*, results from surface contact with glass or other foreign substances. Factor XIIa acts as an enzyme to convert factor XI to the activated form, XIa. Factor IX is activated by enzymatic action of XIa and calcium ions to form IXa. Factor IX interacts with factor VIII, calcium ions, and phospholipid from platelets (PF3) when it becomes activated to enzymatically convert factor X to Xa, its active form. In the *extrinsic system*, tissue thromboplastin (factor III), calcium ions, and factor VII react with factor X, converting it to its active form, Xa. Factor X, now activated, interacts with factor V in the presence of calcium ions and platelet phospholipid to form plasma thromboplastin.

Stage 2—The conversion of prothrombin to thrombin. Plasma thromboplastin with calcium ions converts prothrombin to thrombin.

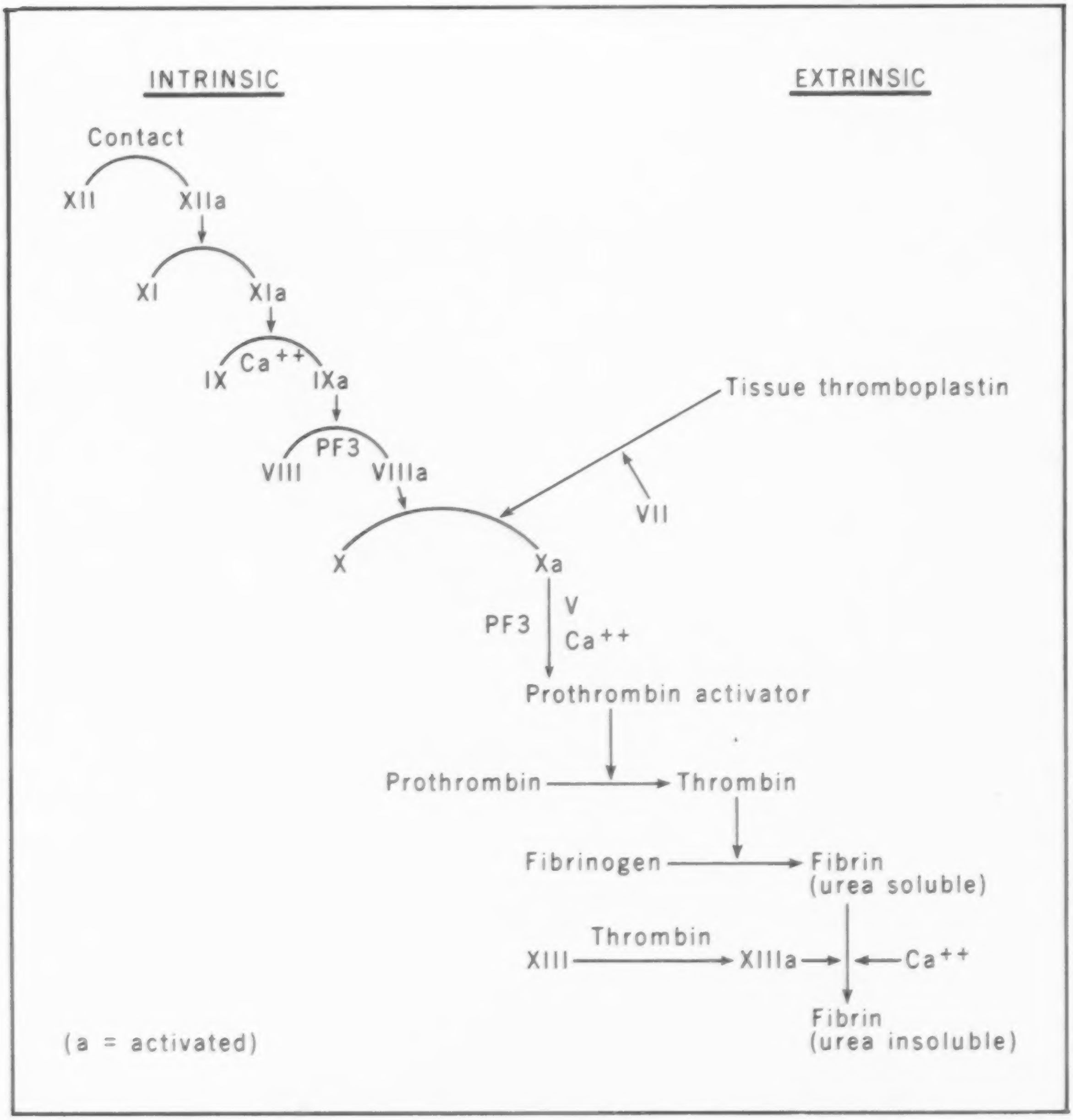
INTRINSIC
EXTRINSIC
Contact
XII
XIIa
XI
XIa
IX
Ca++
IXa
PF3
VIII
VIIIa
Tissue thromboplastin
VII
X
Xa
PF3
V
Ca++
Prothrombin activator
Prothrombin
Thrombin
Fibrinogen
Fibrin
(urea soluble)
Thrombin
XIII
XIIIa
Ca++
Fibrin
(urea insoluble)
(a = activated)

Figure 5-1. Cascade scheme of coagulation (intrinsic and extrinsic process).

Stage 3—The formation of fibrin from fibrinogen. The formation of fibrin occurs by the action of thrombin on fibrinogen. The fibrinogen molecules consist of three polypeptide chains. Thrombin splits off two small peptides on each side of the fibrinogen molecule. (Note fig. 5-2 and 5-3.) The action of thrombin causes the release of fibrinopeptide A from the alpha chain first. Then fibrinopeptide B is released from the beta chain by the action of thrombin to produce the fibrin monomer. These fibrin monomers then polymerize to form a fibrin polymer which is soluble in 5 M (molar) urea. Factor XIII, which is activated by thrombin and calcium, then converts the fibrin polymer to a more stable state by changing the hydrogen bands to covalent bands. The fibrin which results is insoluble in 5 M urea. Subsequent retraction of this clot occurs in the presence of intact platelets. The extrinsic and intrinsic systems are described separately for the purpose of evaluation. Actually, tissue thromboplastin probably activates both systems, since both

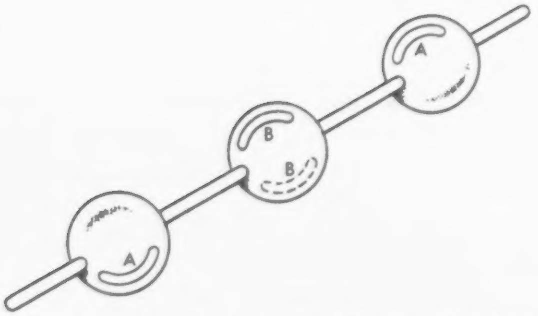

Figure 5-2. Fibrinogen molecules A and B indicate site where peptides are removed as a result of thrombin activity.

the extrinsic and intrinsic mechanism are involved in activating factor X. With the intrinsic system it takes a minimum of 5 minutes, usually more, for fibrin to form. With the extrinsic system, fibrin forms in 12 seconds.

Exercises (059):

1. What are the four components that interact in the presence of calcium to form a clot?
2. List the three stages in the coagulation process.
3. In what possible ways is factor XII activated *in vivo*?
4. In the intrinsic system, with what other constituents does activated factor IX interact to enzymatically convert factor X to Xa?
5. In the extrinsic system, what constituents react with factor X to convert it to its active form, Xa?
6. How is prothrombin converted to thrombin?
7. The formation of fibrin from fibrinogen occurs by the action of what constituent on fibrinogen?
8. In the action of thrombin on the polypeptide chains of the fibrinogen molecule, what process takes place?

060. Name substances in plasma that inhibit clotting and tell how they work; describe anticoagulant therapy in terms of drugs, their effects, and tests used to monitor them.

Inhibitors of the Clotting Mechanism. In addition to the factors necessary for clot formation, plasma contains substances that prevent coagulation. These substances are the naturally occurring or circulating anticoagulants, which may be specific for a single clotting factor or may inhibit a variety of factors.

An inhibitor has been described for virtually every clotting factor. These inhibitors are thought to be responsible for the clotting abnormality found in lupus erythematosus and also for the presence of abnormal protein in macroglobulinemia. The formation of thromboplastin is inhibited by antithromboplastin, which binds factor VIII (antihemophilic globulin) and probably factor IX (plasma thromboplastin component), making them unavailable for the generation of thromboplastic activity. Inhibitors have also been described for factor XII and factor IX, both of which

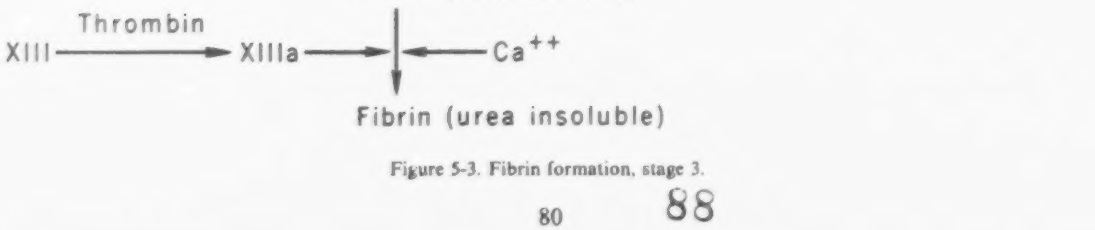

Figure 5-3. Fibrin formation, stage 3.

lose their clot-promoting activity once they have been activated. In addition, inhibitors specific for factor VIII (antihemophilic globulin) and factor IX (plasma thromboplastin component) have been found to develop in some patients deficient in these factors as a result of having received many transfusions.

Anticoagulants. We have two anticoagulants that are used *in vivo* which should be mentioned. First, heparin, which combines with the heparin cofactor, an alpha globulin, to form a potent antithrombin. Heparin itself is a mucopolysaccharide which was originally isolated from the liver. Since heparin cofactor is present in the circulating blood, the injection of heparin intravenously will give an immediate anticoagulant effect. The action of heparin interferes with most laboratory tests because of its antithrombic action. Most commonly we use the coagulation time or the partial thromboplastin time to monitor the heparin therapy. Another anticoagulant is coumadin, which we have heard of previously. This acts as an antivitamin K and will increase the production of normal factor II, factor VII, factor X, and factor IX. The site of action of coumadin is in the liver. Coumadin requires 16 to 24 hours to cause a measurable change in tests such as the prothrombin time. The therapeutic management of coumadin is regulated by the use of the prothrombin time.

Exercises (060):

1. By what substance is the formation of thromboplastin inhibited and how?

2. What type inhibitors have been found to develop in patients deficient in related factors as a result of having received multiple transfusions?

3. How does heparin act as an anticoagulant?

4. What tests are commonly used to monitor the heparin therapy?

5. How does coumadin act as an anticoagulant?

6. By what specific test is coumadin therapy monitored?

061. Indicate whether given statements correctly reflect the tests for laboratory diagnosis of clotting defects.

Laboratory Diagnosis of Clotting Defects. Deficiency of inhibition of any of the clotting factors, except factor VII, may be associated with clinically significant bleeding. Diagnosis of the specific defect in the clotting mechanism cannot be made on the basis of symptomology, but depends on careful laboratory examinations. A scheme of suggested procedures for coagulation studies is given in figure 5-4. Current techniques enable the physician to determine the stage of coagulation impaired, and whether the bleeding is due to the deficiency of a specific clotting factor or factors, an inhibitor of the clotting mechanism, or abnormal fibrinolysis (dissolution of the formed clot).

The activated partial thromboplastin time is the most popular screening method of the detection of blood coagulation disorders. This simple test encompasses all three stages of coagulation and thereby indicates an abnormality in any of the clotting factors, if one exists, with the exception of factor VII and platelets.

In the event that the activated partial thromboplastin time test is abnormal, a more specific testing procedure is required. This method facilitates diagnosis and may be followed by a comprehensive testing program.

To determine stage I deficiencies (formation of thromboplastin), the testing is directed toward: (1) platelet function, and (2) hemophilic factors and Hageman factor (factor XII) deficiencies. In addition to the activated partial thromboplastin time, the prothrombin consumption test and whole blood clotting time are informative in this area. The prothrombin consumption test theoretically measures the amount of unconverted prothrombin present in the serum after a 1-hour clotting period. The whole blood clotting time is an *in vitro* method of observing the coagulation time of the patient, and though quite insensitive, involves all of the coagulation factors. Abnormally long values obtained in either of these tests may be related to platelet malfunction, thrombocytopenia, or one of the hemophilic states. For Hageman factor deficiency, untreated glass and silicone-treated glass whole blood clotting times are compared. This deficiency may be identified by an equally prolonged clotting time in both types of glass.

More confirmatory testing may be made by the activated partial thromboplastin time substitution procedures. Known factor-VIII-deficient plasma is mixed in a 1:1 dilution with the test plasma and tested with the partial thromboplastin time reagent to confirm the diagnosis of hemophilia.

For stage II (conversion of prothrombin to thrombin), the screening test of choice is the prothrombin in time. This test procedure is used primarily for the therapeutic control of coumadin anticoagulant

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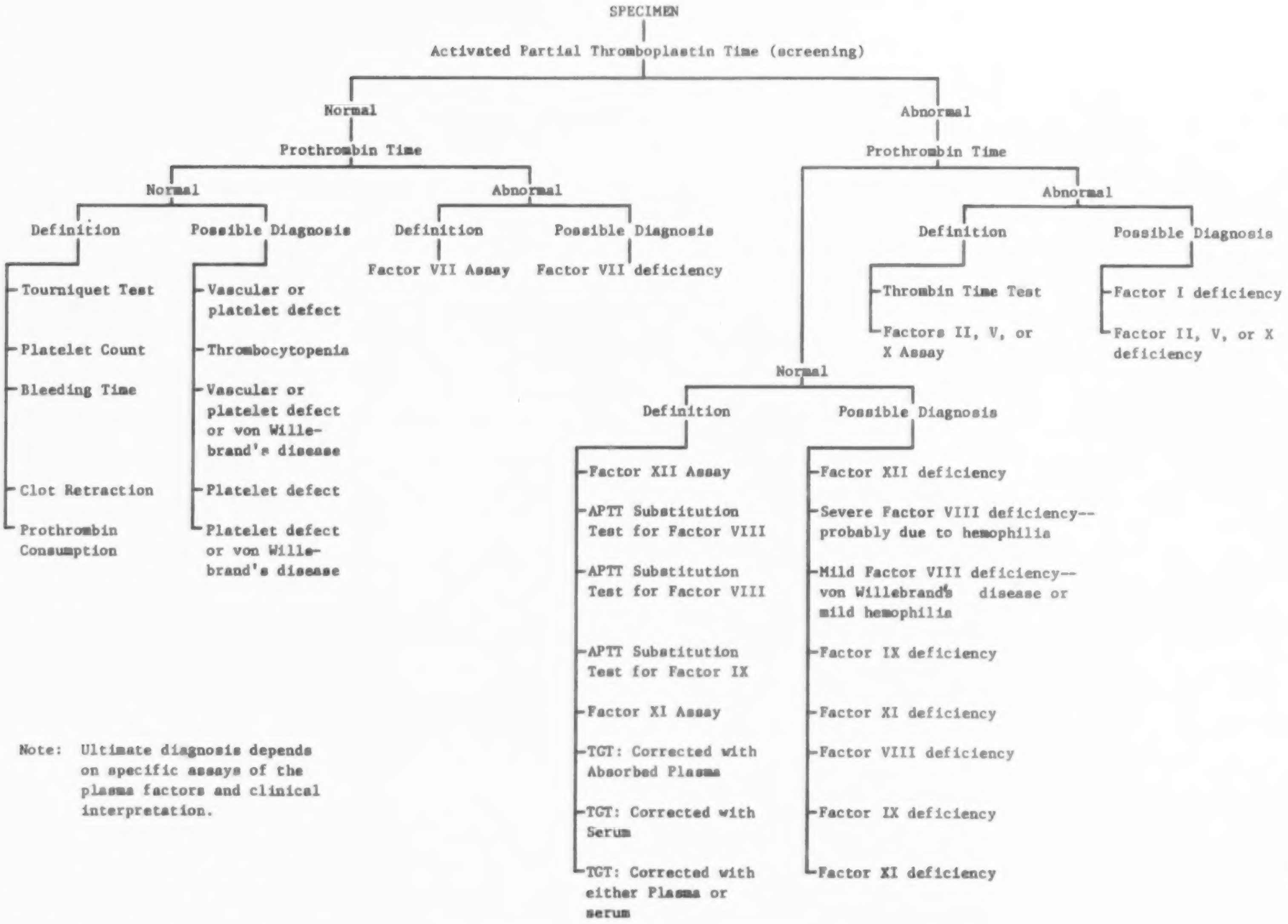
SPECIMEN
Activated Partial Thromboplastin Time (screening)
Normal
Prothrombin Time
Normal
Definition
Tourniquet Test
Platelet Count
Bleeding Time
Clot Retraction
Prothrombin Consumption
Possible Diagnosis
Vascular or platelet defect
Thrombocytopenia
Vascular or platelet defect or von Willebrand's disease
Platelet defect
Platelet defect or von Willebrand's disease
Abnormal
Definition
Factor VII Assay
Possible Diagnosis
Factor VII deficiency
Abnormal
Prothrombin Time
Abnormal
Definition
Thrombin Time Test
Factors II, V, or X Assay
Possible Diagnosis
Factor I deficiency
Factor II, V, or X deficiency
Normal
Definition
Factor XII Assay
APTT Substitution Test for Factor VIII
APTT Substitution Test for Factor VIII
APTT Substitution Test for Factor IX
Factor XI Assay
TGT: Corrected with Absorbed Plasma
TGT: Corrected with Serum
TGT: Corrected with either Plasma or serum
Possible Diagnosis
Factor XII deficiency
Severe Factor VIII deficiency--probably due to hemophilia
Mild Factor VIII deficiency--von Willebrand's disease or mild hemophilia
Factor IX deficiency
Factor XI deficiency
Factor VIII deficiency
Factor IX deficiency
Factor XI deficiency

Note: Ultimate diagnosis depends on specific assays of the plasma factors and clinical interpretation.

Figure 5-4. Suggested procedures for coagulation studies.

therapy. For stage III (formation of fibrin clot), the thrombin time test is used to measure a deficiency of fibrinogen or to detect the inability of the patient's fibrinogen to react with thrombin to form a clot. A delayed reaction may indicate a specific inhibition or a decreased level of fibrinogen.

Exercises (061):
Identify each true statement, and explain why the others are false.

T F 1. The activated partial thromboplastin time encompasses all three stages of coagulation.

T F 2. The activated partial thromboplastin time indicates an abnormality in any of the clotting factors except factor VI and factor VIII.

T F 3. In the event that the activated partial thromboplastin time is abnormal, no further test is required.

T F 4. Testing to determine stage I deficiencies is directed toward platelet function, hemophilic factors, and Hageman factor (factor XII deficiencies).

T F 5. In addition to the activated partial thromboplastin time (APTT), the prothrombin time and bleeding time are informative in this area.

T F 6. The prothrombin consumption test theoretically measures the amount of converted prothrombin present in the serum after a 1-hour clotting period.

T F 7. Known factor-VIII-deficient plasma is mixed in a 1:1 dilution with the test plasma and tested with the activated partial thromboplastin time reagent to conform the diagnosis of hemophilia.

T F 8. For stage II deficiencies, the screening test of choice is the prothrombin consumption time.

T F 9. The prothrombin time measures the functions of factor V (proaccelerin), factor VII (proconvertin), factor II (prothrombin), and factor X (Stuart factor).

T F 10. The prothrombin time is used for therapeutic control of heparin anticoagulant therapy.

T F 11. For stage III deficiencies, the thrombin test is used.

T F 12. The thrombin test is used to detect the inability of the patient's fibrinogen to react with thrombin to form a clot.

062. State the purpose and importance of the fibrinolytic mechanism, and specify the fibrinolytic principle.

Fibrinolysis. The fibrinolytic mechanism provides a means whereby fibrin clots and fibrinous deposits can be removed from the vascular tree. Therefore, the importance of the fibrinolytic system for the prevention of thrombosis is obvious.

A schematic representation of the fibrinolytic mechanism is shown in figure 5-5. The active fibrinolytic principle is called plasmin or fibrinolysin. Plasmin exists in the plasma in the form of an inactive precursor, plasminogen or profibrinolysin. Plasminogen is converted to plasmin by the action of certain activators. Plasmin, once formed, is capable of lysing fibrin clots but may also hydrolyze other substrates as well. The fibrinolytic mechanism is held in check by a system of inhibitors.

Plasminogen exists in two phases: (1) a soluble phase, in which plasminogen circulates in the plasma; and (2) a gel phase, in which the plasminogen is adsorbed to fibrinogen.

The two-phase plasminogen system explains how fibrinolytic activity *in vivo* results in localized and well-contained lysis of both thrombi and fibrinous deposits without the deleterious effects of generalized and systemic hyperfibrinolysis. Soluble-phase plasminogen refers to plasminogen circulating in the blood

ACTIVATION

PLASMINOGEN (PROFIBRINOLYSIN) → PLASMIN + PEPTIDES (FIBRINOLYSIN)

PROTEOLYSIS

PLASMIN + FIBRIN & OTHER PROTEINS → SPLIT PRODUCTS

INHIBITION

PLASMIN + ANTIPLASMIN (ANTIFIBRINOLYSIN) ⇌ INACTIVE COMPLEX

Figure 5-5. Schema of the human fibrinolytic mechanism.

with other plasma proteins. Gel phase plasminogen, on the other hand, represents plasminogen adsorbed to fibrin. Plasminogen is closely associated with fibrinogen and tends to coprecipitate with the latter substance.

When plasminogen is in the soluble phase, it can be activated to plasmin and circulate freely in the plasma. Under conditions of slow plasminogen activation, small amounts of plasmin may appear in the circulation, but generalized fibrinolysis and fibrinogenolysis do not occur because antiplasmin is capable of effectively neutralizing low concentrations of plasmin. However, with rapid and fulminant activation of plasminogen, such as might occur in certain diseases or following thrombolytic therapy, circulating plasmin concentrations may reach values high enough to overcome antiplasmin inhibition. In this circumstance, proteolysis of circulating fibrinogen and other susceptible plasma substrates occurs. As a result, hypofibrinogenemia occurs and increased levels of proteolytic products of fibrinogen or fibrinogen from fibrin split products are found in the circulation. Because these changes may result in a hemorrhagic tendency, this phenomenon has been called pathologic proteolysis.

When plasminogen is in the gel phase, physiologic proteolysis occurs. Physiologic proteolysis is the mechanism thought to explain how thrombi might be lysed without dissemination of the fibrinolytic activity. Since plasminogen is incorporated into fibrin clots, a close spatial relationship exists between fibrinogen and plasminogen within thrombi. Plasminogen activators are able to diffuse into the clot and to convert plasminogen to plasmin. Since plasmin is formed only inside the clot, it is not susceptible to inhibition by antiplasmin in the circulating plasma. Thus clot lysis may occur, but the disseminated effects of plasmin are prevented. The hypothesis of physiologic proteolysis is attractive, but alternative explanations are possible.

Much evidence suggests that the coagulation and the fibrinolytic systems are in equilibrium. Generally, when fibrinolysis is increased, coagulation is also increased.

Exercises (062):

1. What purpose does the fibrinolytic mechanism serve?
2. What is the importance of the fibrinolytic system?
3. What is the active fibrinolytic principle called?
4. In what form does plasmin exist in the plasma?
5. What are the two phases in which plasminogen exists?
6. Why do generalized fibrinolysis and fibrinogenolysis *not* occur under conditions of slow plasminogen activation?
7. Why are fibrinolysis and coagulation believed to be in equilibrium?

063. In terms of inherited clotting deficiencies, briefly compare the two types of hemophilia, and cite genetic similarity and the deficient factors associated with the disease.

Inherited Deficiencies in the Clotting System. The most familiar bleeding disorder is hemophilia. Many recurrent bleeding disorders have been erroneously attributed to this disease. Classic hemophilia (hemophilia A, factor VIII deficiency) is now distinguishable from the more recently recognized hemophilia B (factor IX deficiency). Both diseases are inherited as sex-linked recessive characteristics. Since the trait is carried on the X chromosome, this explains the presence of the disease in males. The daughters of a hemophilic man (married to a normal female) will be carriers of the recessive gene. The sons will be normal. In rare instances, a female hemophiliac has been identified. This unusual occurrence is attributed to the mating of a male hemophiliac with a female hemophilic carrier.

Hemophilia varies in its severity. Mild forms of the disease are asymptomatic. Diagnosis for the identification of hemophilia A or B is important from the standpoint of treatment. Hemophilia B is the rarer form of the disease, and therapy by transfusion is more successful because of the greater stability of the factor IX in stored blood.

Deficiencies of factor XI (plasma thromboplastin antecedent) and factor XII (Hageman trait) are not inherited as sex-linked characteristics, but the genes are carried on the autosomes, and therefore both sexes are affected. The roles of factor XII (Hageman factor) and coagulation are interdependent. It is suggested that activated factor XII in turn activates factor XI in the early part of the coagulation process and subsequently participates in the coagulation mechanism.

The laboratory followup of therapy control in these stage I deficiencies may easily be followed by using the partial thromboplastin time test as a guide.

Exercises (063):

1. What is the difference between hemophilia A and hemophilia B?

2. Genetically, how are both hemophilia A and B similar?

3. Why is hemophilia more common in males than in females?

4. Which form of hemophilia is the rarer form?

5. Why is transfusion therapy of this rarer form more successful?

6. Why are both sexes affected in the deficiencies of factor XI (plasma thromboplastin antecedent) and factor XII (Hageman factor)?

064. List some causes of acquired deficiencies in blood coagulation and the tests most often used for study of patients suspected of having coagulation disorders.

Acquired Deficiencies in Blood Coagulation. Hemorrhagic complications may arise at any step of the coagulation process. Clinical manifestations are sometimes multiple and may be due to one or a combination of disorders. Both platelet and plasma factors may be affected. Some of the major causes of acquired deficiencies are: liver disease, hyperfunction of the spleen, hypersensitivity to drugs with demonstrable immunologic reaction, the septicemias, bone marrow hypoplasia, mass transfusions, and circulating anticoagulants.

Diagnostic blood coagulation tests most often used for a study on a patient suspected of having a coagulation disorder are the activated partial thromboplastin time test, platelet count, bleeding time, prothrombin time, and clotting time. A prothrombin consumption test also may be carried out, and the clot of the clotting time may be kept for observation of retraction or lysis.

The tests above are the usual preliminary or screening tests performed in studying suspected defects in the coagulation mechanism as diagrammed in figure 5-4. On the basis of the results of these tests, the physician may make modifications in the patient's coagulation mechanism by giving the patient anticoagulation therapy. The course of such therapy is usually evaluated with one of the tests for coagulation deficiency discussed in the following section. The results of the preliminary screening tests may result in requests for tests specific for individual components that cause coagulation deficiencies.

Exercises (064):

1. List some major causes of acquired blood coagulation deficiencies.

2. What are the tests most often used in the study of suspected coagulation disorders?

5-2. Tests for Coagulation Deficiencies

When coagulation disorders are suspected, a number of tests are performed in order to detect or identify the cause of the disorder. Further, certain assay procedures are necessary to follow the course of therapy in conditions of known etiology. A prothrombin time is a good example of the latter. We will briefly discuss each of the major tests performed in the typical clinical laboratory. Refer to table 5-2 as you read about each of these tests for an indication of their significance to the physician in evaluating coagulation disorders.

065. Point out the purpose of the tourniquet test, and name conditions associated with abnormal results.

Tourniquet Test. In some bleeding disorders, capillary fragility is increased when the capillaries are subjected to increased positive pressure. This may be accomplished by placing a sphygmomanometer cuff on the arm of the patient. The cuff is inflated to a point midway between the systolic and diastolic pressure. After 5 minutes the cuff is removed. One to 2 minutes after this, the arm is examined for petechiae. The number of petechiae appearing on a representative 1 square centimeter area of the arm or back of the hand may be reported. Another method of reporting is to draw a circle about the size of a 25-cent-piece and to count the petechiae within the circle (0 - 10 = 1+; 10 - 20 = 2+; 20 - 50 = 3+; over 50 = 4+). Petechiae which appear only just below the cuff are not considered significant. In positive tests they appear over the entire arm and hand.

Increased vascular fragility is sometimes found in qualitative and quantitative platelet abnormalities, vitamin C deficiency, and in the various purpuras. The term "purpura" is not specific, but applies to a number of affectations characterized by bleeding into tissue. The tourniquet test is most often performed by the physician, and may be required in the clinical laboratory.

Exercises (065):

1. What does the tourniquet test measure?

TABLE 5-2
RESULTS OF LABORATORY TESTS IN
VARIOUS COAGULATION DISORDERS

Coagulation Disorder	Coagulation Test									
	TT	BT	PT	PC	PTT	CT	CR	TGT	Plt. ct.	Fib.
Vascular defect	+	+		+				±		
Thrombocytopenia	+	±		+			+	±	+	
Thrombocytopathia	±	±		+			+	+		
Deficiency of:										
Prothrombin			+		+					
Factor V			+	+	+			+		
Factor VII			+							
Factor VIII				+	+	+		+		
Factor IX				+	+	+		+		
Factor X			+	+	+	+		+		
Factor XI				+	+	+		±		
Factor XII				+	+	+		±		
Hypofibrinogenemia			+			+	+			+
Presence of fibrinolysin										+

TT = Tourniquet test
BT = Bleeding time
PT = Prothrombin time
PTT = Partial thromboplastin time
+ = Abnormal
± = May be abnormal

PC = Prothrombin consumption
CT = Clotting time
CR = Clot retraction
TGT = Thromboplastin Generation test

Plt. ct. = Platelet count
Fib. = Fibrinogen assay

2. What are some conditions associated with increased capillary fragility?

066. Indicate whether given statements correctly reflect the principle of the bleeding time, the dependent factors, differences in the two given methods, and conditions associated with abnormal values.

Bleeding Time—Duke Method. The bleeding time is the time it takes for a small standardized wound to stop bleeding. It is dependent upon the elasticity of the skin and capillary vessels, the efficiency of tissue fluids, and the mechanical and chemical action of the platelets.

Turning our attention to the bleeding time, there are two methods at present popularly used. The Duke method is the older of the two methods. This test is performed on the lobe of the ear preferentially, since the fingertips vary as to the thickness of the skin. This variance is usually related to the type of work regularly performed. The charge has been made that it is difficult to apply pressure to the ear lobe when the bleeding time is markedly prolonged; although this can be inconvenient, complications have been minimal. If the lobe of the ear is extremely flabby and the skin rather loose, we find that we sometimes have difficulty getting the values to be reproducible.

In doing the Duke bleeding time, cleanse the ear lobe gently—you don't want to stimulate the circulation by rough handling—and then, using a disposable lancet, make a 2- to 4-mm cut. By using disposable materials, you avoid the possibility of contaminating the patient with the hepatitis virus. Blot the blood every 30 seconds, using filter paper, and note the time when bleeding has ceased. Although the normal range is 1 to 3 minutes, values up to 4 minutes seem to be a "gray zone," and one would not like to use these values as a diagnostic criterion. When an abnormal value is obtained, the test should be done on the other ear.

Bleeding Time—Ivy Method. With the Ivy method, the forearm is used. Put the blood pressure cuff on the arm and inflate it to a pressure of 40 mm of mercury. With this pressure maintained, cleanse the forearm gently and choose a site where there are no visible vessels. Make a cut 2 to 4 mm in depth and touch the drop of blood every 30 seconds, using filter paper. The normal in this method is 2 to 6 minutes. If you are doing a number of repeat bleeding times, this method has a distinct advantage as far as having a satisfactory area available. Also, it is easier to do the Ivy bleeding time when working with children. Abnormalities of the bleeding time occur with platelet defects, such as thrombocytopenia and the thrombopathies, especially if the patient has taken aspirin. Abnormal values are also seen in patients with von Willebrand's disease. This, you might recall, is a bleeding disorder which has an autosomal dominant inheritance pattern and the individual has a diminished factor VIII activity. These people usually have an abnormal bleeding time after ingestion of aspirin, but not consistently. Other individuals have a normal factor VIII activity but have a response to aspirin. These may be referred to as vascular dysfunctions to distinguish them from von Willebrand's patients.

Sources of Error. Despite that fact that there are intrinsic limitations to the bleeding time, it is a valuable test when carefully performed. Nevertheless, the technician must be aware of sources of error in the performance of the test.

An inadequate puncture results in a poor flow of blood. The site must not be squeezed to obtain a free flow of blood. Low skin temperature produces a constriction of the capillary vessels, resulting in decreased blood flow. A standardized puncture is necessary for valid results. Too deep a wound prolongs the bleeding time, while a shallow wound shortens the bleeding time.

Choice of Procedure. The Ivy bleeding time is the method of choice because the blood pressure on the vessels is constant, the incision is uniform, and the arm offers an area for multiple determinations.

The bleeding time depends primarily on extravascular and vascular factors and, to a lesser degree, on the factors of coagulation. The chief factor controlling bleeding from a small cut is the constriction of the minute vessels following injury. Accuracy in this test is enhanced by blotting the drops of blood at shorter intervals of time as the drops of blood become progressively smaller.

Thrombocytes play an important part in the formation of the hemostatic plug which seals off a wound. In thrombocytopenic purpura there is a decrease in platelets, resulting in a prolonged bleeding time due to a defective platelet plug. An additional factor that prolongs the bleeding time in this condition is a defect in capillary contraction.

In hemophilia the bleeding time is normal. This is explained by the fact that there are no vascular or extravascular abnormalities. However, the test should not be performed on a known hemophiliac, for delayed oozing of blood is a real hazard.

Exercises (066):

Identify each true statement in the following exercises, and explain why the others are false.

T F 1. The bleeding time is the time it takes for any puncture wound to stop bleeding.

T F 2. The bleeding time is dependent upon the elasticity of the skin and arterioles, the efficiency of tissue structure, and the mechanical and chemical action of the platelets.

T F 3. In the Duke method for bleeding time, values may be more reproducible if the ear is extremely flabby and the skin rather loose.

T F 4. In doing the Duke bleeding time, you should stimulate the circulation by slightly rough handling.

T F 5. The finger is punctured 2 to 4 mm in depth in the Ivy method for bleeding time.

T F 6. Abnormalities in the bleeding time are noted with platelet defects, such as thrombocytopenia and thrombopathies, especially if the patient is taking aspirin, and in von Willebrand's disease.

T F 7. Squeezing the site of the puncture is permissible to obtain a free flow of blood for the bleeding time.

T F 8. Low skin temperature produces a constriction of the capillary vessels, resulting in decreased blood flow.

T F 9. Too deep a wound may shorten the bleeding time, while a shallow wound prolongs it.

T F 10. The Duke bleeding time is the method of choice, because the blood pressure on the vessels is constant and the incision is uniform.

T F 11. The bleeding time depends primarily on the factors of coagulation rather than on extravascular and vascular factors.

T F 12. In hemophilia the bleeding time is normal.

T F 13. The bleeding time should not be performed on a known hemophiliac.

067. State the basic principle of the coagulation time of whole blood; the dependent factors in the procedures, the deficiencies associated with prolonged value, and sources of error.

Coagulation Time of Whole Blood (Lee and White). The whole blood clotting time is a rough measure of all intrinsic clotting factors in the absence of tissue factors. Variations are wide and the test sensitivity is limited. Coagulation of blood in this procedure involves mostly the production of the prothrombin activator, plasma thromboplastin. Only a few seconds are then required to convert prothrombin to thrombin, and fibrinogen to fibrin, as noted in figure 5-6. These moderate deficiencies in stages 2 and 3 of the coagulation process will not significantly prolong the clotting time. The coagulation time is mainly influenced by deficiencies in stage 1 of the clotting process. Moderately severe hemophilia due to a factor VIII deficiency, afibrinogenemia, and severe fibrinolytic conditions will cause a prolonged clotting time. When heparin is administered as a therapeutic anticoagulant in the treatment of thromboembolic disorders, its effect may be monitored by the degree of prolongation of the coagulation time. The therapy may then be changed to the coumarin drugs and monitored by means of the prothrombin time. Despite the fact that heparin is faster acting than coumarin drugs, the therapy is more difficult to maintain.

Sources of errors. The clotting time can be altered by mechanical factors. Chief among these are temperature variations, test tube size, surface (coated or uncoated, wet or dry, smooth or rough), volume of blood in the tube, and the frequency as well as the way in which the tubes are tilted. The normal range depends upon how this test is performed.

The clotting time may be decreased by rough handling of the blood specimen, presence of tissue fluids (traumatic venipuncture), frequent tilting of the tube, and unclean tubes.

Increase in the clotting time may be a result of extreme increases in temperature, variation in pH, and performance of the test at room temperature.

It is important to place exactly 1 ml of whole blood in each tube. Quantities greater than 1 ml will prolong the clotting time. Less than 1 ml of blood in the tube shortens the clotting time. Bubbles entering the syringe when the blood sample is taken will increase the coagulation rate.

Discussion of procedure. The coagulation time is normal in thrombocytopenic purpura. This is explained by the fact that only a small number of thrombocytes need be present for normal coagulation to take place.

It is suggested that at least one tube remain in the water bath at 37° C to be observed at 1, 2, and 4 hours for clot retraction; also, that the tube remain in the water bath overnight and be checked the next day for abnormal clot lysis, erythrocyte fallout, and complete clot retraction. Normal values are:

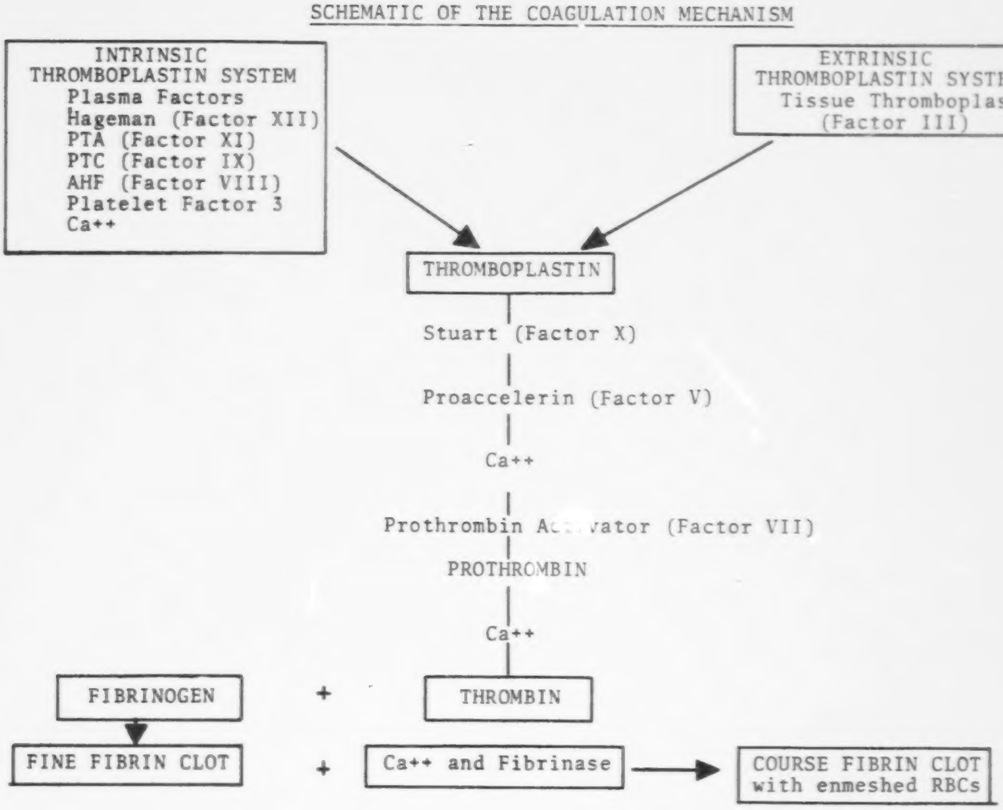
SCHEMATIC OF THE COAGULATION MECHANISM
INTRINSIC THROMBOPLASTIN SYSTEM
Plasma Factors
Hageman (Factor XII)
PTA (Factor XI)
PTC (Factor IX)
AHF (Factor VIII)
Platelet Factor 3
Ca++
EXTRINSIC THROMBOPLASTIN SYSTEM
Tissue Thromboplastin (Factor III)
THROMBOPLASTIN
Stuart (Factor X)
Proaccelerin (Factor V)
Ca++
Prothrombin Activator (Factor VII)
PROTHROMBIN
Ca++
FIBRINOGEN
+
THROMBIN
FINE FIBRIN CLOT
+
Ca++ and Fibrinase
COURSE FIBRIN CLOT with enmeshed RBCs

Figure 5-6. Schematic of the coagulation mechanism.

a. Glass tubes; 5 to 15 minutes at 37° C, and up minutes at room temperature.
b. Polystyrene or siliconized tubes: 25 to 45 minutes.
c. Activated clotting time: Up to 2 minutes 15 seconds.

Exercises (067):

1. What does the whole blood clotting time measure?

2. Primarily, what activator is produced in this coagulation process, and what other processes follow?

3. By deficiencies in what stage of the clotting process is the coagulation time mainly influenced?

4. What abnormal coagulation conditions may cause a prolonged coagulation time?

5. When heparin is administered as a therapeutic anticoagulant, what possible disorders are being treated?

6. When the therapy is changed to coumarin drugs, what procedure is used to monitor the therapy?

7. What are some mechanical factors which may alter the clotting time?

8. List some factors which may decrease the clotting time.

9. List some factors which may increase the clotting time.

10. What effect does thrombocytopenic purpura have on the coagulation time (increased, decreased, normal, none)? Why?

11. After completing the Lee and White clotting time, you are requested to allow one tube to remain in the water bath and observe it for 1, 2, and 4 hours before discarding. What will you be observing the tube for?

12. Why should you also allow the tube to remain in the water bath overnight, and what two brief test procedures will you be checking?

068. Indicate whether given statements correctly reflect the primary deficiency measured by the clot retraction, the clot retraction procedure, and the principle and procedure for the screening test for fibrinolysis.

Clot Retraction and Fibrinolysis. Whole blood, having clotted, normally retracts from the sides of the container. This reaction is a function of the quantity and quality of intact platelets, the fibrinogen content of the plasma, and the ratio of plasma volume to red cell mass. Dissolution of the clot due to the action of proteolytic enzymes then ensues.

Clot retraction procedure. The standard procedure is to place a tube containing blood in a water bath and observe for retraction of the clot at the end of 1, 2, 4, and 24 hours. A semiquantitative technique may be performed by measuring the amount of serum expressed. This may be related to the total volume and reported in percent according to the formula:

$$\frac{\text{Volume of expressed serum}}{\text{Volume of whole blood}} \times 100$$

Normally, 45 to 60 percent of the serum is expressed from the clot, and this will occur within 1 to 2 hours.

Poor clot retraction occurs in thrombocytopenia, qualitative platelet deficiency, and in cases of an increase in the red cell mass. Clot retraction may appear to be increased in severe anemia and extreme hypofibrinogenemia, because of the formation of a small clot due to the relative increase in plasma volume.

Screening test for fibrinolysis. The process of clot dissolution, or *fibrinolysis,* is a necessary activity following clot formation. Once the clot has served its purpose, it must be removed. The mechanism of clot dissolution is very complex, as discussed earlier in objective 062. Through a series of activators and precursors, plasminogen, a widely distributed globulin, is converted to *plasmin.* (Another term for plasmin is *fibrinolysin.*) Plasmin acts locally to enzymatically dissolve the clot. Several major coagulation defects relate to fibrinolysis. Consequently, it is a matter of laboratory importance to evaluate fibrinolytic activity.

The fibrinolytic activity of a patient's plasma may be semiquantitatively measured by adding the test plasma to normal plasma. After clotting, the mixture is observed for zones of lysis. In performing this test, prepare mixtures of patient's plasma, normal plasma, and thrombin, as shown in table 5-3. Place the tubes in a water bath and examine them hourly for lysis. If necessary, allow incubation to continue overnight. In the presence of an abnormally high level of lysin, lysis will be observed within several hours. An approximation of lysin concentration may be determined by observing the ratio of patient to normal plasma. Tubes 1 and 6 are the normal and the patient's plasma controls, respectively. If the clots in all the tubes remain solid and well formed, the test is considered negative. Bacterial contamination must be avoided.

Exercises (068):

Indicate whether each of the following statements is true or false, and correct those that are false.

T F 1. The retraction of whole blood that has clotted is a function of the quality of fibrinogen and thrombin, and the ratio of plasma volume to red cell mass.

T F 2. The standard procedure for clot retraction is to place a tube containing blood in a water bath and observe for retraction of the clot at the end of 1, 2, 4, and 24 hours.

T F 3. A semiquantitative technique may be performed for clot retraction by measuring serum absorbed.

T F 4. Hypofibrinemia is the primary deficiency measured by the clot retraction.

TABLE 5-3
TABLE OF PLASMA RATIOS FOR FIBRINOLYSIS SCREENING TEST

Tube	1	2	3	4	5	6
Normal Plasma (ml)	1.0	0.8	0.6	0.4	0.2	0
Patient's Plasma (ml)	0	0.2	0.4	0.6	0.8	1.0
Thrombin (ml)	0.1	0.1	0.1	0.1	0.1	0.1

T F 5. Poor clot retraction occurs in thrombocytosis and in cases of a decrease in red cell mass.

T F 6. The process of clot dissolution is called fibrinolysis.

T F 7. Once the clot has served its purpose, it must be removed.

T F 8. Through a series of activators and precursors, plasminogen is converted to antiplasmin.

T F 9. Antiplasmin acts locally to enzymatically dissolve the clot.

T F 10. In performing the semiquantitative test for fibrinolytic activity, you would prepare mixtures of patient's plasma, normal plasma, and thromboplastin.

T F 11. If clots in all the tubes remain solid and well formed, the test is considered positive.

069. From a list of statements about the reagents, procedures, equipment, and sources of errors for platelet counts, identify the true statements and explain why the others are not correct.

Platelet Count. Platelet counts are of great significance as an aid in the diagnosis of bleeding disorders. You may recall that platelet function is primarily the stoppage of bleeding (hemostasis) by plugging the capillary walls to inhibit bleeding and maintaining a seal of the capillary walls.

The fact that platelets are very small, disintegrate easily, and are hard to distinguish from dirt make them very difficult to count. They adhere readily to one another and easily become attached to any foreign body. EDTA used as an anticoagulant helps to decrease the clumping of platelets.

Results are significantly lower and less satisfactory when fingertip, toe, or heel is used than when platelet counts are performed on venous blood.

Direct Methods. Three methods for direct platelet counting are (1) the Rees-Ecker method, (2) the Brecker-Cronkite method, which employs the use of the phase microscope, and (3) the electronic cell counter procedure.

Rees-Ecker method. In this method, a sample of blood is diluted with an isotonic anticoagulant containing brilliant cresyl blue, which stains the platelets a light bluish color. In addition, the solution serves as a fixing diluent. After mixing, the specimen is introduced into the counting chamber and the number of platelets in a known volume is counted.

The Rees-Ecker diluting fluid contains 3.8 g sodium citrate, 0.2 ml 40 percent formaldehyde, 0.1 g brilliant cresyl blue, a quantity diluted to the mark in a 100 ml volumetric flask. This procedure is outlined in AFM 160-51, *Clinical Laboratory Procedures—Hematology.* With proper adjustment of the light and continual focusing, the platelets are seen as blue, highly refractile bodies, which may be round, oval, or comma shaped. They vary in size from 1 to 5 micrometers.

Since two chambers are used, the volume of both chambers is 0.2 mm^3 (area × depth = 2 sq mm × 0.1 mm = 0.2 mm^3. The calculation is as follows:

$$\frac{\text{Total platelets counted} \times \text{dilution (200)}}{\text{Volume (0.2)}} = \text{platelets per mm}^3$$

Sources of errors. The count is invalid if clumps of platelets are noted in counting. There should be an even distribution of platelets. The range of errors is estimated to be 16 to 25 percent. Once the diluted count has been removed from the shaker, it should not be allowed to stand more than 8 to 10 seconds without being remixed. Further, the blood should be diluted and smears made within 5 hours of blood collection, or within 24 hours if the blood is refrigerated. Because of the extremely small size of blood platelets, it is a common error to confuse them with yeasts, debris, and precipitated stain.

Brecker-Cronkite Method. Whole blood is diluted with 1 percent ammonium oxalate, which completely homolyzes the red cells. The platelets are counted, using the phase hemacytometer and phase contrast microscope. The platelets appear round or oval, pink, purple, or even black under a phase condenser. The *Unopette* No. 2708 is recommended as more convenient and far superior for quality control purposes than the RBC pipette. In this unit, 13 μl of blood is diluted in 1.3 ml of 1 percent ammonium oxalate (1:100). Fill two unopettes with patient's sample and allow to stand for a minimum of 10 minutes. Then fill a clean, flat, bottom-phase counting chamber—one unopette on each side. If the red blood cell diluting pipette is used according to AFM 160-51, the blood is drawn to the 1 mark and the 1 percent ammonium oxalate to the 101 mark (1:100). It is important with phase microscopy that the diluent be optically clear; that is, free from dirt, dust, etc. If such contaminants are present, they may be quickly removed by filtering the diluent through a membrane filter. Thin hemacytometers designed for the purpose are covered with a Nr. 1 cover glass instead of the standard hemacytometer cover glass. Place the hemacytometer on wet filter paper and cover it with a petri dish cover for 15 minutes to allow the platelets to settle. Count the platelets in all 25 squares of the area used for the RBC count and obtain the average of the two counts from both sides of the counting chamber. The count from either side should not deviate more than 10 percent from the other count. The average of the two counts is multiplied by 1000. The unopette has a method for use without the phase microscope, in which case the manufacturer's instructions must be followed.

Platelet counts by this method are considerably more accurate than by the Rees-Ecker method. The method of blood collection reduces platelet agglutination, fragmentation, and disintegration. The use of ammonium oxalate diluent clears the background by hemolysis of the red cells. The use of phase contrast microscopy overcomes the difficulty in distinguishing platelets from extraneous particles. Technically, the method is less difficult and time consuming than the less accurate Rees-Ecker method. The only disadvantage is that it requires specialized equipment. For those laboratories that are equipped, or that can be equipped, with a phase contrast microscope, this method is strongly recommended.

Results of both methods should be doublechecked by examination of the platelets on a Wright-stained smear. The presence of platelet clumps in the hemacytometer chamber indicates that incipient clotting has occurred. This may result from difficulties in entering the vein, delay in anticoagulating the blood, or delay in diluting the blood with ammonium oxalate solution when capillary blood is diluted directly. The presence of platelet clumps precludes reliable counts, and a fresh sample must be collected. If the pipettes are allowed to rest for even 10 seconds after shaking, they must be reshaken before loading the chambers.

The normal range for both given methods is 150,000 to 350,000/mm^3. However, it must be realized that there is a great variance in normal values, which differ with each technique, laboratory, and technician.

Exercises (069):

Indicate whether each of the following statements is true or false, and correct those that are false.

T F 1. When the fingertip, toe, or heel is used for platelet counts, instead of venous blood, the results are not significantly affected.

T F 2. In the Rees-Ecker method, the sample is diluted with an isotonic anticoagulant containing new methylene blue.

T F 3. The anticoagulant in the Rees-Ecker solution is 3.8 g of ammonium oxalate.

T F 4. The count is considered invalid if clumps of platelets are noted in counting.

T F 5. The range of error in platelet counts is estimated to be 1.6 to 2.5 percent.

T F 6. The blood should be diluted and smears made within 5 hours of blood collection, or within 24 hours if the blood is refrigerated.

T F 7. In the Brecker-Cronkite method, the blood is diluted with 1 percent ammonium citrate, which completely hemolyzes the red cells.

T F 8. In the Brecker-Cronkite method, platelets appear round, or oval, pink, purple, or even black under a phase condenser.

T F 9. When the count from both sides of the counting chamber is obtained, the deviation between the two counts must not exceed 20 percent.

T F 10. Platelet counts by the Brecker-Cronkite method are considered less accurate than the Rees-Ecker method.

T F 11. The use of phas contrast microscopy does very little to overcome the difficulty of distinguishing platelets from extraneous particles.

T F 12. Results of all platelet counts should be doublechecked by examination of the platelets on a Wright-stained smear.

T F 13. The presence of platelet clumps precludes reliable counts, and a fresh sample must be collected.

070. Point out basic procedures, principle, and sources of error in the electronic platelet count, and state the procedure for the indirect platelet count.

Direct Platelet Count—Electronic Counting. Platelet counts may be performed by the following automated methods: Coulter Counter, Technical Hemolab, Fisher Autocytometer, and MK-4 Platelet Counter. As an example, let's discuss the use of the Coulter Counter for platelet counts. The red cells must first be removed from the blood sample by one of the following three procedures: (1) sedimentation method, providing separation of red cells from platelet-rich plasma in 10 to 50 minutes; (2) closely controlled centrifugation (300 g for 5 minutes), providing reproducible separation of red cells without significant loss of platelet from plasma; and (3) test tube held in vertical position in a modified table top centrifuge and spun at 40 g for 25 seconds, producing rouleaux, which settle rapidly to yield platelet-rich plasma in 2 minutes.

An aliquot of plasma is diluted with Isoton®. The count obtained is then corrected for coincidence and dilution. The surface change of the RBCs will trap a small portion of plasma, leading to an increased concentration of platelets in the plasma. The excess concentration is a function of the hematocrit. Thus, the count is further corrected for the hematocrit to obtain a true platelet count.

Sources of error. Platelet-rich plasma should be obtained without losing platelets or having too many red cells remain after centrifugation. Careful technique is important in making the microdilution, and the cleanliness of the related glassware and aperture tube of the counter should be maintained. An excessive number of red cells in the plasma will give falsely low counts, because platelets entering the aperture at the same time as red cells will not be detected. High WBC counts will give a falsely low platelet count. Platelets as large as red cells will be screened out by the upper threshold, also yielding a falsely low count. If the sample is hemolyzed or the red cell fragments are present, the count is falsely elevated.

The automated platelet count may be performed on various models of the Coulter Counter: models A, B, F, Fn, and ZBI. In addition, their thrombocounter is designed exclusively for platelet counts. As in the previously mentioned manual methods, a Wright-stained mean must be examined before reporting the count.

Indirect platelet count. Thrombocytes are counted indirectly on a stained blood smear and expressed in number per 1000 red blood cells. The number of platelets per mm^3 can be easily estimated if you know the red blood cell count and how many platelets there are per 1000 red blood cells:

$$\frac{\text{RBCs/cmm} \times \text{platelets per 1000 RBCs}}{1000} = \text{thrombocytes per mm}^3$$

If fewer red blood cells are counted, the formula is adjusted accordingly, with some loss of accuracy. This method should not be attempted without some type of ocular disc to narrow the field of vision.

Exercises (070):

1. In order to obtain platelet-rich plasma for platelet counts by the Coulter Counter method, one of three procedures is used. Briefly describe these procedures.

2. What corrections are made in the platelet count?

3. How will excessive numbers of red cells in the plasma give a falsely low count?

4. How will the count be affected when the sample is hemolyzed or red cell fragments are present?

5. What two factors are used to estimate the indirect platelet count?

071. Point out the significance, procedures, reagents, normal values, and sources of errors of the test for fibrinogen deficiency.

Fibrinogen Deficiency Test. You may recall that fibrinogen is a protein which is produced by the liver and is used in the clotting of blood. During the process, the fibrinogen is converted to fibrin. The fibrinogen level in the blood may be measured by the fibrinogen deficiency test. The test is used mainly in the diagnosis and treatment of the hemorrhagic diseases. It is also useful during surgical hemorrhage or obstetrical bleeding and is sometimes ordered as an emergency procedure. Some conditions accompanied by a deficiency of fibrinogen are: congenital afibrinogenemia, congenital hypofibrinogenemia, severe liver damage during or following surgery, complications during pregnancy, abortion, metastatic carcinoma, severe burns, and intravascular clotting.

Methods of testing for Fibrinogen Deficiency. The test for fibrinogen deficiency may be performed by screening and quantitative methods.

Screening methods. The screening methods are rather simple and are generally performed by obtaining blood or plasma, adding a reagent, and inspecting the preparation for agglutination and clotting. A rough estimation of fibrinogen by the screening methods is sufficient for most clinical purposes. Two commonly used screening methods are (1) the Warner-Lambert method and (2) the Fi-Test method.

The Warner-Lambert screening method for fibrinogen deficiency is done by preparing plasma and adding physiological saline, adding this mixture to a solution of calcium and thromboplastin, observing the formation of a clot, and recording the time. In this procedure, the calcium and thromboplastin are contained in the simplastin reagent. The reagents of this test must be absolutely free of fibrinolysin. If not, the clot will be partially or totally dissolved and erroneous results will be reported.

The Hyland FI-TEST® is a rapid slide screening test providing a simplified bedside or operating room procedure for screening hypofibrinogenemia. The test requires 1 drop of heparinized whole blood obtained by finger prick and a reagent prepared from polystyrene latex. Using a capillary pipette, a drop of heparinized blood is transferred to one of the bottles containing glycine-saline buffer diluent and mixed. Two drops of latex antihuman fibrinogen reagent are added to the drop of diluted blood specimen and to the diluted normal control in a separate oval on the same slide. Rapid and clean-cut reactions are obtained to distinguish normal plasma fibrinogen levels from abnormally low levels (below 100 mg/dl). Agglutination in a degree that is comparable to that shown by the normal control is normal. (Normal plasma levels are from 250 to 400 mg/dl.) No agglutination indicates hypofibrinogenemia or plasma levels of 100 mg/dl or less.

Quantitative methods. The quantitative methods are more involved and are usually done in the chemistry department. These methods include the titration procedure, colorimetric procedure, and the use of the coagulation analyzer method. We shall limit our discussion to the colorimetric method because it is the most common method used in most laboratories.

In the colorimetric procedure (semiquantitative), the fibrinogen assay presented in AFM 160-51 is based on the principle that fibrinogen is salted out by ammonium sulfate and measured with a spectrophotometer. Reagents used are Parfentjev reagent, which contains ammonium sulfate, NaC1, and merthiolate in distilled water.

The Parfentjev reagent deteriorates after 2 weeks of storage and must be prepared fresh every 2 weeks. Outdated prepared standards should not be used because the fibrinogen has a potency of 60 months. Normal values are 200–400 mg/dl/

Exercises (071):

1. For what general purposes is the fibrinogen deficiency test used?

2. In the Warner-Lambert screening method for fibrinogen, to what reagent is the plasma-saline solution added before observing for the formation of a clot?

3. How does the presence of fibrinolysin in the reagents affect the test?

4. In the Fi-Test method for fibrinogen screening, two drops of what reagent are added to 1 drop of diluted blood specimen and normal control?

5. In the semiquantitative method for fibrinogen assay, the fibrinogen is salted out with what reagent?

6. How often should the Parfentjev reagent be made to insure reliability of the reagent?

072. Describe the prothrombin time test in terms of its significance, reagents, procedures, sources of errors, and guidelines for quality control.

One-Stage Prothrombin Time (Quick). When optimal amounts of thromboplastin, calcium, and citrated plasma are mixed under carefully controlled conditions, fibrin strands will normally form within a matter of seconds. The time required for the fibrin to appear is known as the one-stage prothrombin time. This test measures prothrombin and accessory factors. The prothrombin time of a patient's plasma may be expressed as a percentage of the control plasma and reported as *percent activity*. The test is based on having an optimum concentration of calcium ions and an excess of thromboplastin, the only variable being the concentration of prothrombin and accessory factors in a carefully controlled volume of plasma. As you may recall from the schematic of the coagulation mechanism in figure 5-6, with tissue thromboplastin, the clotting time depends on the concentration of prothrombin, factor V, factor VII, and factor X (assuming fibrinogen and anticoagulant activity to be normal). The prothrombin time is essentially a test of the extrinsic pathway of clotting. In the presence of calcium, the factors listed act on prothrombin to form a fibrin clot. The test is not sensitive to factor IX and other factors involved in intrinsic thromboplastin formation.

Reagents and procedure. Since factor V is labile in oxalated blood, sodium citrate, in which factor V is relatively stable, should be used as the anticoagulant. If the test cannot be performed immediately, the specimen should be refrigerated. The reagents are: (1) citrated plasma, normal control plasma, and patient plasma; and (2) commercial thromboplastin-calcium chloride reagent. The sample of blood is conveniently obtained in a vacutainer tube containing 0.5 ml of 3.8 percent sodium citrate. The blood is centrifuged promptly at 2,000 r/min. Immediately remove the plasma to a 12 x 75 mm test tube and refrigerate until ready to use. Prepare thromboplastin-calcium chloride as prescribed. Incubate the thromboplastin, patient plasma, and control plasma for 5 minutes. Plasma may not be incubated for more than 10 minutes. Blow 0.1 ml of warm plasma into a test tube containing 0.2 ml of thromboplastin-calcium chloride mixture and simultaneously start stopwatch. Incubate at 37° C for 5 to 6 seconds; then remove tube. Tilt gently back and forth against a diffused light until the very earliest fibrin strand can be detected. All tests should be run in duplicate.

In order to maintain good quality control, the use of an automatic instrument to perform prothrombin time tests is highly recommended, since it is more reliable than doing the test with test tubes, pipettes, and a stopwatch. This is especially so if several technicians perform these tests on a rotating schedule. One such instrument, a fibrometer, is illustrated in figure 5-7. An automatic dispensing pipette that dispenses both patient specimen and thromboplastin is seen at the bottom of the picture. The main unit in the picture is composed of two components: a series of incubator wells on the right and the actual timing device with more incubator wells on the left. Preincubated patient plasma is added to a plastic cup in the well under the center post seen just above the electrical socket. The automatic dispenser is then filled with preincubated thromboplastin. The dispenser is reset so that the clot timing device is actuated when the plunger is depressed. When the clot timer is actuated, the center post drops down so that a wire loop and a wire needle come into contact with the plasma-thrombin mixture. When a large enough clot has formed to simultaneously contact both loop and needle, the timing device stops. The prothrombin time is read directly from the digital readout register on the lower end of the timing device.

Sources of error. Follow the procedures listed below to avoid the more common sources of error.

(1) If an automatic timer is not used, it should be remembered that the time is critical. The stopwatch must be started the instant the plasma contacts the thromboplastin-calcium chloride reagent and must be stopped immediately when the clot forms.

(2) The thromboplastin must be prepared as stated in the manufacturer's directions.

(3) Avoid traumatic venipuncture. Tourniquet application must not be prolonged.

(4) The test must be accomplished within 3 hours after collection. Factor V is labile and will decrease in activity as the blood or plasma stands. Refrigerated plasma is stable for a maximum of 4 hours.

(5) The test must be performed at the temperature of 37° C.

(6) Hemolyzed plasma must not be used.

(7) The plasma may be frozen and stored up to 1 week without any significant effect on the prothrombin time.

Interpretation. The prothrombin activity is reported in seconds as well as in percent by applying the patient's prothrombin time in seconds to a prothrombin activity curve. Prolongation of the one-stage prothrombin time does not measure prothrombin deficiency but rather indicates some failure of conversion of prothrombin to thrombin. Specifically, the test detects deficiencies of factor I, II, V, VII, or X. Reductions in any one or combination of these factors prolong the one-stage prothrombin time. The normals are 12 to 15 seconds. Drugs that may shorten the prothrombin time by interacting with anticoagulants

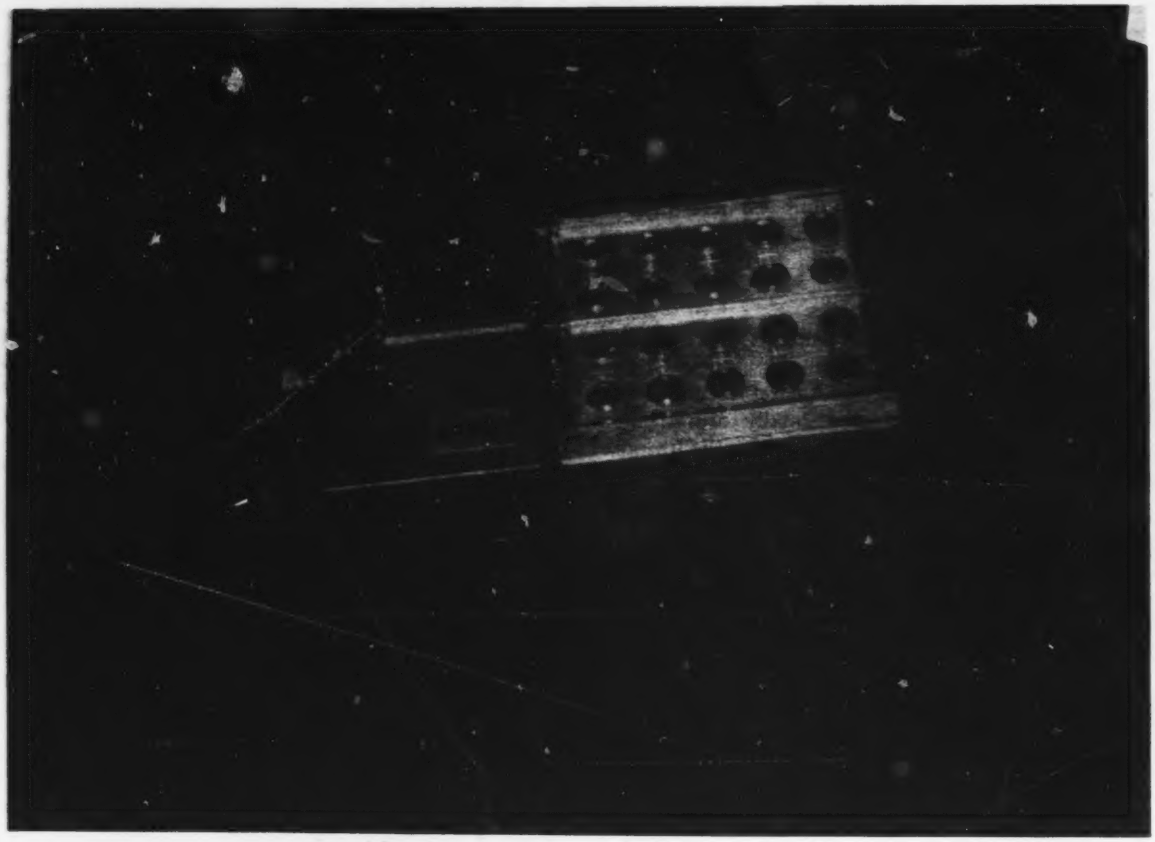

Figure 5-7. Fibrometer.

include barbiturates, digitalis, diuretics, vitamin K, and oral contraceptives.

Quality control of prothrombin time. Two prothrombin time ranges should be established—the therapeutic range and the normal range. Run the patient plasma and control plasma in duplicate. Control values should fall within ±0.5 seconds of each run. The control results should be charted daily to provide a constant check on test conditions and their accuracy. The use of an automatic clot timer is strongly recommended, since it allows to a great extent for standardization and uniformity independent of the time of day, the day of the week, or the technician's skill.

Exercises (072):

1. The prothrombin time is based upon the optimum concentration of what cation?
2. If the optimum concentration of the cation is present, what is then the variable in a carefully controlled volume of plasma?
3. Prothrombin time is a test of which clotting pathway?
4. Why is the use of sodium oxalate not recommended for the prothrombin time?
5. If the prothrombin time cannot be performed immediately, what must be done with the plasma?
6. What is the maximum time of incubation for the plasma?
7. What factor will decrease activity as the blood or plasma stands?

8. The prothrombin time detects deficiencies in what factors?

9. What drugs may shorten the prothrombin time by interacting with the anticoagulant?

10. How close should control values fall between each run?

11. Why is the use of an automatic clot timer recommended?

073. Indicate whether given statements correctly reflect the principle, reagents, method, and interpretation of the prothrombin consumption test.

Prothrombin Consumption Test. Intrinsic thromboplastin converts all plasma prothrombin to thrombin during the normal clotting process. When intrinsic thromboplastin is deficient, only a portion of prothrombin will be converted, leaving residual prothrombin in the serum. The prothrombin consumption test measures the residual prothrombin in the serum. The remaining prothrombin is inversely proportional to the amount of intrinsic thromboplastin generated during coagulation. The amount of thromboplastin is dependent on the concentration of factors VIII, IX, and platelet factor 3 (adequacy of platelets).

Reagents. The reagents used are (1) Simplastin A®, (2) patient's serum, and (3) control serum. In the test, the serum supplies factors VII, IX, X, XI, and XII. The Simplastin A® contains the tissue thromboplastin, calcium, factor V, and fibrinogen. Simplastin A® may be obtained through local purchase supply channels from General Diagnostics Division, Warner Lambert Laboratories.

Method. The procedure with Simplastin A® is accomplished by the following method:

(1) Draw the blood into a glass tube (not siliconized) and allow the blood to incubate for 2 hours at 37° C after clotting to insure the complete inactivation of the thrombin.

(2) Remove the serum from the clot after the 2-hour incubation and store at 4° C until used. The serum must not be held for more than 2 hours at 4° C.

(3) Perform the one stage prothrombin assay, substituting the Simplastin A® for the thromboplastin-calcium and the serum for the plasma. Run the test in duplicate, and run a normal control each time.

Interpretation and Normal Values. Serum prothrombin times over 20 seconds are normal with Simplastin A®. Times of 18 to 20 seconds are usually considered normal, and times less than 18 seconds are abnormal. Each laboratory should establish its own normal range. NOTE: A decreased value indicates a deficiency in factors VIII and IX and platelets or the presence of a circulating anticoagulant.

Exercises (073):
Indicate whether each of the following statements is true or false, and correct those that are false.

T F 1. During the normal clotting process, intrinsic thromboplastin converts all plasma thrombin to prothrombin.

T F 2. The remaining prothrombin in the prothrombin consumption test is inversely proportional to the amount of intrinsic thromboplastin generated during coagulation.

T F 3. The amount of thromboplastin is dependent upon the concentration of factors VII and X and platelet factor 3 or adequacy of platelets.

T F 4. The Simplastin A® supplies factors VII, IX, X, XI, and XII.

T F 5. Patient's serum supplies the tissue thromboplastin, calcium, factor V, and fibrinogen.

T F 6. Siliconized tubes may be used in the given procedure for prothrombin consumption test.

T F 7. Two-hour incubation of the blood at 37° C after clotting insures complete inactivation of the thrombin.

T F 8. After removal of the serum from the clot, the serum may be stored indefinitely at 4° C.

T F 9. For the prothrombin consumption test, the one-stage prothrombin assay may be performed, substituting the Simplastin A® for the thromboplastin-calcium and serum for plasma.

T F 10. Serum prothrombin times over 20 seconds are normal with Simplastin A®.

T F 11. A decreased value indicates a deficiency in factors VIII and IX and platelets or the presence of a circulating anticoagulant.

074. State the principle, the purpose, the reagents, the procedure, sources of error, dependent factors for the normal values, and interpretation of the activated partial thromboplastin time test (APTT).

Activated Partial Thromboplastin Time (APTT). The APTT is the most useful single procedure available for routine screening of coagulation disorders. All coagulation factors except factors VII and XIII and platelet factor 3 are assayed by the APTT.

Principle. The patient's plasma contains all intrinsic factors for clotting except calcium, which is bound by the anticoagulant, and platelets, which are removed by centrifugation. Calcium and a platelet factor reagent or a partial thromboplastin are added to the patient's fresh plasma. The clotting time is recorded. An activator is added to make the activation of the plasma independent of the surface of the tube.

Reagents. The reagents used are (1) calcium chloride, (2) kaolin suspension, (3) normal control plasma, and (4) partial thromboplastin. The reagent may be obtained as Thrombofax® from Ortho Diagnostics, Platelin® from Warner-Lambert Laboratories, or Dade's Activated Cephaloplastin®.

Procedure. The activated procedure is used in preference to the regular PTT. With the regular PTT, the degree of activation is controlled by the conditions under which the plasma is prepared, such as the condition of the glassware (rough surface), time in contact with the glass, and type of syringe used. However, with the APTT, complete activation is assured by the addition of kaolin, an activator to the *Platelin*® or *Celite*® to plasma when using Thrombofax®. No addition of an activator is necessary in the procedure with Dade's Activated Cephaloplastin. The procedures will vary, depending on the method used, manual or automated. Nevertheless, a constant, adequate incubation time should be established for maximum sensitivity and reproducibility. The plasma may be refrigerated (up to 3 hours) until ready for testing. The following brief manual technique may be modified slightly when the Fibrometer or any other automated instrument is used:

(1) Pipette 0.1 ml of Platelin® with activator or Dade's Activated Cephaloplastin® into a tube.

(2) Add 0.1 ml of the patient's plasma to the tube and mix by giving the tube a sharp shake. (A 1:1 ratio of plasma and *Celite*® reagent is required with Ortho procedure.)

(3) After 2 to 4 minutes of activation, transfer 0.1 ml of 0.02 M calcium chloride to the mixture. Simultaneously start the second timer. Keep the calcium chloride at 37° C for a minimum of 5 and a maximum of 60 minutes.

(4) Immediately insert the nichrome wire loop into the tube, moving it across the bottom every 2 seconds.

(5) At the first appearance of fibrin strands, stop the timer and record the time.

Repeat the test on another aliquot of the specimen and report the average of the two tests. Run the control twice. As mentioned previously, performance of the test on the fibrometer or any other automated instrument insures better quality control.

Sources of error. The following sources of error should be observed:

(1) Plasma must be prepared with care without disturbing the buffy coat.

(2) If plasma is left at room temperature, the test must be performed within 45 minutes. Otherwise, plasma can be stored at 4° C for 3 hours.

(3) Clean glassware and accurate pipetting are essential for valid results.

(4) Do not use sodium oxalate as an anticoagulant. Sodium citrate is a better preservative and activates plasma faster.

(5) Tubes must be shaken thoroughly before and after incubation to insure adequate mixing.

Interpretation. Circulating anticoagulants will prolong the APTT. They include heparin, lupus erythematosus anticoagulants, fibrin degradation products, fibrinolysins, and antibody types of anticoagulants such as antifactor VIII antibody.

APTT for control of heparin therapy. For many years the therapy of heparin was controlled by the use of the Lee-White method of whole blood coagulation. The method is often considered difficult to standardize and time consuming. Animal research and experimentation has shown that the APTT is less time- consuming and easier to standardize. Animal experiments have been confirmed by clinical experience.

Normal values. The normal range varies from laboratory to laboratory, depending on the anticoagulant used to obtain the plasma, the type of equipment used, and the individual running the test. Considering these variables, the suggested normal range is 28 to 45 seconds. An APTT of 50 seconds or more usually is indicative of a defect in the coagulation mechanism.

Exercises (074):

Complete the following statements:

1. All coagulation factors, except factors ________, ________, and ________ are assayed by the APTT.
2. The patient's plasma for the test contains all intrinsic factors except ________, which is bound by the anticoagulant, and ________, which are removed by centrifugation.
3. In order to make the plasma independent of tube surface an ________ is added.
4. The reagents used are ________, ________, ________, and ________.
5. The regular PTT is controlled by such conditions as ________, ________, and ________.
6. With the APTT, complete activation is assured by the addition of ________ to the Platelin®.
7. In the given procedure, the reagents are added in the following order: 0.1 ml ________, 0.1 ml ________, and 0.1 ml ________.
8. If plasma is left at room temperature, the test must be performed within ________ minutes.
9. When removing plasma from tube, avoid disturbing the ________.
10. Three circulating anticoagulants which may prolong the APTT are ________, ________, and ________.
11. Compared to the Lee-White method of whole blood coagulation, the ________ is less time consuming and easier to standardize when used in the control of heparin therapy.
12. The normal range may vary from laboratory to laboratory, depending on ________, ________, and ________.

075. Indicate whether given statements correctly reflect the principle, the purpose, the reagents used, and interpretation of the thromboplastin generation test (TGT).

Thromboplastin Generation Test (TGT). The thromboplastin generation test measures the efficiency with which plasma thromboplastin is formed. A potent thromboplastin is generated when platelets, prothrombin-free plasma, serum, and calcium are mixed. After the generation of the thromboplastin, all factors necessary to produce a clot are present except for factor I and factor II. When these factors are added to a normal thromboplastin mixture, a clot is detected within 7 to 16 seconds. With an abnormal time, deficient factors are identified by substituting adsorbed patient plasma or aged patient plasma. The test is able to detect factor VIII and factor IX deficiencies and is able to distinguish between the two. Factors XI and XII may also be detected, but cannot be differentiated from each other. A platelet abnormality may be detected when the patient's platelets are used in the test. A procedure is outlined in AFM 160-51.

Reagents. The reagents used in the test include the following:

(1) Normal plasma substrate is used as a control, and serves as a source of factors I, II, V, VII, and X.

(2) Partial thromboplastin serves as a platelet-like substance.

(3) Normal adsorbed plasma reagent serves as a source of factors, V, VIII, XI, and XII. Chemically pure barium sulfate is used in the plasma absorption procedure.

(4) Normal aged serum.

(5) Calcium chloride, 0.025 M.

(6) Patient's adsorbed plasma reagent. This reagent is adsorbed with barium sulfate.

(7) Patient's aged serum reagent. This reagent is also adsorbed with barium sulfate.

Interpretation. Results of the thromboplastin generation test are interpreted in terms of each of the reagents used; for example, substrate plasma, adsorbed plasma, serum, and platelets. A defect in the adsorbed plasma indicates a factor V or factor VIII abnormality. If factor V is decreased, the prothrombin time should be prolonged. An abnormality in the serum fraction suggests a factor IX or X deficiency. If factor X is decreased, the prothrombin time should be prolonged. Finally, if the adsorbed plasma and serum are abnormal, factors XI and XII are considered. Failure to obtain any degree of correction when normal reagents are substituted may indicate an inhibitor directed against thromboplastin formation. These results are listed in table 5-4.

Exercises (075):

Indicate whether each of the following statements is true or false, and correct those that are false.

T F 1. The thromboplastin generation test measures the efficiency with which extrinsic thromboplastin is formed.

T F 2. After thromboplastin is generated, all factors necessary to produce a clot are present except factors III and IV.

T F 3. When the TGT is abnormal, deficient factors are identified by substituting adsorbed patient plasma or aged patient plasma.

T F 4. The TGT is able to detect factor XI and factor XII deficiencies, and is able to distinguish between the two.

TABLE 5-4
RESULTS OF THE THROMBOPLASTIN GENERATION TEST IN VARIOUS DISORDERS

Coagulation abnormality	Reagent		
	Adsorbed plasma	Serum	Platelets
Factor V	Abnormal	Normal	Normal
Factor VII	Normal	Normal	Normal
Factor VIII	Abnormal	Normal	Normal
Factor IX	Normal	Abnormal	Normal
Factor X	Normal	Abnormal	Normal
Factor XI	Abnormal	Abnormal	Normal
Factor XII	Abnormal	Abnormal	
Inhibitor	Abnormal	Abnormal	Normal
Thrombocytopathia	Normal	Normal	Abnormal

T F 5. The test is unable to distinguish between factors VIII and IX, but both are well detected.

T F 6. A platelet abnormality may be detected when the patient's platelets are used.

T F 7. Normal plasma substrate is used as a control in the TGT and serves as a source of factors V, VIII, XI, and XII.

T F 8. Normal adsorbed plasma reagent used in the TGT serves as a source of factors I, II, V, VII, and X.

T F 9. Chemically pure barium sulfate used in the plasma adsorption procedure serves as a platelet-like substance.

T F 10. A defect in the adsorbed plasma indicates a factor V or factor VIII abnormality.

T F 11. An abnormality in the serum fraction suggests a factor IX or X deficiency.

T F 12. Failure to obtain any degree of correction when normal reagents are substituted indicates an inhibitor directed against thromboplastin formation.

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Answers for Exercises

CHAPTER 1

Reference:

001 - 1. c.
001 - 2. h.
001 - 3. i.
001 - 4. k.
001 - 5. g.
001 - 6. j.
001 - 7. a.
001 - 8. f.
001 - 9. d.
001 - 10. e.
001 - 11. a.
001 - 12. d.
001 - 13. b.
001 - 14. k.
001 - 15. j.

002 - 1. F. Precursor and mature cells as a single, though discontinuous organ.
002 - 2. F. Changes in tissue oxygen tension within the kidney.
002 - 3. T.
002 - 4. T.
002 - 5. F. It is not well understood and breakdown is not as readily observable.
002 - 6. T.
002 - 7. T.
002 - 8. T.
002 - 9. F. Particularly the spleen.
002 - 10. F. Iron is used again.
002 - 11. T.
002 - 12. F 160 to 415 mg/dl.
002 - 13. F. Glucose.

003 - 1. The exchange of gases between the alveoli of the lungs and the bloodstream.
003 - 2. Nitrogen, oxygen, carbon dioxide, and water vapor.
003 - 3. The value of nitrogen is constant.
003 - 4. Air pressure is reduced by the pressure of the water vapor.
003 - 5. At the point where the alveolar pressure reaches 100 mg Hg, which is also the partial pressure of oxygen within the capillary.
003 - 6. The difference between the two pressures results in an exchange of carbon dioxide into the alveoli.
003 - 7. Carbon dioxide has a greater coefficient of diffusion than oxygen.
003 - 8. Normal sea level pressure: 760 mm Hg.
Water vapor: 47, 760 - 47 = 713 mm Hg.
003 - 9. Neither the atmospheric composition nor the action of carbon dioxide and oxygen in the alveoli is static.
003 - 10. T.
003 - 11. F. Gases will flow from a high pressure area to one of lower pressure.
003 - 12. F. Figure 1-2, 90 percent.
003 - 13. T.

004 - 1. The exchange of gas between the blood and the tissue cells.
004 - 2. Oxygen, 100 mm Hg, and carbon dioxide, 40 mm Hg.
004 - 3. Lower. While the transfer of oxygen is occurring, there is also a transfer of carbon dioxide. The constant metabolic activity of the cell results in a high production of carbon dioxide.
004 - 4. Arterial blood contains more oxygen than does venous blood.
004 - 5. No; the pulmonary artery contains venous blood which is en route to the lungs.
004 - 6. Hemoglobin is a complex molecule of rather high molecular weight (64,500 amu). It contains 4 atoms of iron surrounded by chains of amino acids.

005 - 1. Hemoglobin oxidizes to the ferric (+3) state and thus become oxyhemoglobin. In giving up oxygen, oxyhemoglobin reduces to the ferrous state.
005 - 2. Energy.

006 - 1. f.
006 - 2. h.
006 - 3. e, g.
006 - 4. c.
006 - 5. j.
006 - 6. e.
006 - 7. e.
006 - 8. a.
006 - 9. a, c.
006 - 10. b.
006 - 11. c.
006 - 12. d.
006 - 13. d.
006 - 14. d.

007 - 1. Atria.
007 - 2. Arteries.
007 - 3. Oxygenation.
007 - 4. Right; superior vena cava; inferior vena cava.
007 - 5. Tricuspid.
007 - 6. Pulmonary valve; pulmonary artery.
007 - 7. Atrium; veins.
007 - 8. Mitral.
007 - 9. Aortic; aorta.
007 - 10. Coronary; portal.
007 - 11. Coronary.
007 - 12. Portal.

008 - 1. F. 90 to 140 mm Hg.
008 - 2. F. 60 to 90 mm Hg.
008 - 3. T.
008 - 4. T.
008 - 5. F. Evans blue dye method and ^{51}Cr have been replaced by the simultaneous method not dependent on the hematocrit.
008 - 6. T.
008 - 7. T.
008 - 8. F. It does not differ significantly.

008 - 9. F. They will not until tissue fluids move into the vascular system or until patient received fluids intravenously.
008 - 10. T.

CHAPTER 2

009 - 1. F. Same.
009 - 2. T.
009 - 3. F. Poor.
009 - 4. F. Lymphocytes.
009 - 5. T.
009 - 6. F. Greater chance; blade should be used with great caution on the infant's heel.

010 - 1. Avoid harm to the patient.
010 - 2. Veins may become distended and easier to enter by allowing the arm to hang down for 2 or 3 minutes, by massaging the blood vessel toward the body, or by gently slapping the site of puncture.
010 - 3. Obtain assistance from a more experienced technician or consult a physician.
010 - 4. Under no circumstances should a technician draw blood from a jugular vein or femoral vein.
010 - 5. Changes may occur in the concentration of blood components.
010 - 6. Rolling veins may be held firm by placing the thumb on the vein so that a 1 or 2 inch length of vein lies between the thumb and the puncture site.
010 - 7. Release the tourniquet, promptly withdraw the needle, and apply pressure to the puncture site.
010 - 8. It is acceptable to puncture the veins 3 or 4 inches below the site of the I.V.
010 - 9. Raising the arm upward will slow blood flow and minimize leakage at the puncture site.
010 - 10. Within 3 hours.

011 - 1. a, c, d, e.
011 - 2. e.
011 - 3. b.
011 - 4. b.
011 - 5. c.
011 - 6. b.
011 - 7. c.
011 - 8. d.
011 - 9. a.
011 - 10. d.
011 - 11. c.
011 - 12. c.

012 - 1. Hemolysis may result.
012 - 2. Warming the Hayem's solution to 60° C will counteract the effect of cold agglutinins without hemolyzing the red blood cells. Use 0.85 percent NaCl or Glower's solution.
012 - 3. $\frac{.3}{(101 - 1)} = \frac{.3}{100} = 1{:}333$
012 - 4. In cases of high red count; for example, polycythemia.
012 - 5. Blood drawn beyond the mark deposits a thin film of cells and can produce an elevated cell count.
012 - 6. It prevents rouleaux and clumping of the red cells.
012 - 7. ±30 percent.

013 - 1. f.
013 - 2. e.
013 - 3. d.
013 - 4. i.
013 - 5. b.
013 - 6. a.
013 - 7. h.
013 - 8. c.

014 - 1. Dirty pipettes and counting chamber and contaminated diluting fluid.
014 - 2. $60 \times \frac{\text{dilution (10)} \times \text{depth (10)}}{\text{area (4)}}$
$= 1{,}500\ \text{WBC}/\text{mm}^3$
014 - 3. Red blood cell diluting pipette; dilution factor of 1:100.
014 - 4. 15 percent.
014 - 5. $15{,}000 \times \frac{100}{100 + 50}$
$= 15{,}000 \times \frac{100}{150}$ or 2/3
$= 10{,}000/\text{mm}^3$.
015 - 1. Tube Nr. 3
015 - 2. Fresh or crenated.
015 - 3. $\frac{9 \times 10 \times 1}{9} = 10$ cells per mm^3.
015 - 4. To prevent clumping of WBCs.
015 - 5. Centrifuge, make a smear, and stain with Wright stain.
015 - 6. The predominant type of cell is an indication of the disease process. For example, infections due to bacteria produce an increase in the neutrophils.

016 - 1. The sperm count varies considerably in different portions.
016 - 2. Preferably within 30 minutes and never over 2 hours.
016 - 3. Time collected and time received in the laboratory.
016 - 4. The action of fibrinolysin.
016 - 5. On arrival (15 to 30 minutes), 3 hours, and 6 hours.
016 - 6. This impairs motility.
016 - 7. Sodium bicarbonate—5 gm; formalin—1 ml; distilled water—100 ml.
016 - 8. It stops the sperm from moving so that they can be accurately counted and, in addition, dissolves the mucus.
016 - 9. 127 × 20 × 5 × 10 × 1000 = 127,000,000/ml.
016 - 10. 60-150 million/ml.
016 - 11. Wright or Giemsa stains.

017 - 1. c, d.
017 - 2. a, b.
017 - 3. c.
017 - 4. c.
017 - 5. b.
017 - 6. b.
017 - 7. e.

018 - 1. f.
018 - 2. e.
018 - 3. d.
018 - 4. a.
018 - 5. a.
018 - 6. b.
018 - 7. g.
018 - 8. c.
018 - 9. o.
018 - 10. m.
018 - 11. p.
108 - 12. j.
018 - 13. k, l.
018 - 14. k.
018 - 15. l.
018 - 16. h.
018 - 17. h.
018 - 18. i.
018 - 19. r.

019 - 1. Dilution inaccuracies, false impulses, and contaminating particles as a source of false impulses.
019 - 2. No more than 10 minutes.
019 - 3. 30 minutes.
019 - 4. 100 per mm^3 or less.
019 - 5. They will be counted as cells.
019 - 6. A plugged orifice or contaminating debris.
019 - 7. 12-15 seconds.
019 - 8. A dirty orifice or dirty manometer and mercury.
019 - 9. If the level is too high, it may be drawn into the vacuum pump, which may become damaged.
019 - 10. Once a week.

020 - 1. c.
020 - 2. c.
020 - 3. e.
020 - 4. j.
020 - 5. i.
020 - 6. f.
020 - 7. g.
020 - 8. h.
020 - 9. i.
020 - 10. k.

021 - 1. Mechanical, optical, electronic, and data presentation.
021 - 2. Cyanmethemoglobin method.
021 - 3. The hematocrit is measured, and the MCV, MCH, and MCHC are calculated.
021 - 4. They scatter and absorb light when the illumination is an intense, direct, straight-line light source.
021 - 5. It is measured. The measure can be used to count and size particles.
021 - 6. The optical.
021 - 7. The loss of light that does not continue along the fixed plane (extinction) is measured.
021 - 8. It generates electrical pulses having magnitude relative to the measured optical property.
021 - 9. The sensor signal is fed into a series of electronic counters and register. The measured information is processed and stored until it is ready to be printed.
021 - 10. Diluted and repeated. Limitation range is 3-23 g/dl.

022 - 1. Incorrect labeling of patient souce and sample number.
022 - 2. Both will be erroneous.
022 - 3. The MCV is not affected.
022 - 4. A flase low leukocyte count.
022 - 5. Pulses from electrical apparatus, flickering fluorescent lamps, and sparking motor brushes.
022 - 6. Leukemia cells may disintegrate in the counter, causing a falsely low leukocyte count.

023 - 1. T.
023 - 2. F. Platelet quantity and quality also evaluated.
023 - 3. F. Last. Used to doublecheck the white cell count and estimate roughly the hematocrit, hemoglobin, and red blood cell count.
023 - 4. c.
023 - 5. c.
023 - 6. b.
023 - 7. d.
023 - 8. e.
023 - 9. a.
023 - 10. c.
023 - 11. b.
023 - 12. d.
023 - 13. e.
023 - 14. a.

024 - 1. F. 1 week.
024 - 2. F. Blood smears should not be routinely made from blood containing anticoagulants because the anticoagulants may distort cells and alter staining.
024 - 3. F. You should not blow air on the slide to enhance drying. This may distort the cells.
024 - 4. F. 95 percent methanol.
024 - 5. T.
024 - 6. T.
024 - 7. F. Leukopenia.
024 - 8. T.

025 - 1. Supra-vital.
025 - 2. Methylene blue and eosin dissolved in methyl alcohol.
025 - 3. It fixes the blood film to the slide.
025 - 4. It should cover the slide, and not be allowed to remain long enough to evaporate.
025 - 5. Blowing gently on the slide.
025 - 6. The buffering stage.
025 - 7. Heat darkens the stain and may cause cell distortion.
025 - 8. The slides will break.
025 - 9. They control the amount of solution delivered to each slide.
025 - 10. Longer.

026 - 1. d.
026 - 2. c, e.
026 - 3. e.
026 - 4. c.
026 - 5. a.
026 - 6. a.
026 - 7. a.
026 - 8. a.
026 - 9. c, e.
026 - 10. a.
026 - 11. b.
026 - 12. a. (Ref. table 2-2)
026 - 13. f. (Ref. table 2-2)

027 - 1. a. What is the size of this cell?
b. What are the features of the nucleus?
c. What are the features of the cytoplasm?
027 - 2. Use an ocular micrometer.
027 - 3. The cytoplasm mass is twice that of the nuclear mass.
027 - 4. It decreases.
027 - 5. Lymphocytes.
027 - 6. The smear and staining technique.
027 - 7. a. Physical (poor technique with the spreader in making the smear).
b. Chemical (dirty or damp slides).
027 - 8. The presence, absence, type, and quantity of granules.
027 - 9. In thick smears their normal nuclear configuration may be distorted by the pressure of erythrocytes.
027 - 10. If the nucleus is more indented than bean-shaped, the cell is a band.
027 - 11. If filaments are present, the cell is a neutrophil.
027 - 12. The presence, absence, and number of nucleoli in the nucleus.

028 - 1. F. Oil immersion.
028 - 2. F. Normal.
028 - 3. F. Monocytes and granulocytes clump near edges, and lymphocytes remain in the central portion.
028 - 4. T.
028 - 5. T.
028 - 6. F. 3 instead of 4; count lesser figure for cells that are relatively rare.
028 - 7. T.
028 - 8. F. 10,000 to 13,000.
028 - 9. T.
028 - 10. F. The estimation *should not* be substituted for more accurate and precise counts.

029 - 1. h.
029 - 2. g.
029 - 3. d.
029 - 4. e.
029 - 5. c.
029 - 6. e, f.
029 - 7. b.
029 - 8. b.
029 - 9. a, i.
029 - 10. a.
029 - 11. a.
029 - 12. a.

030 - 1. Crystal clear.
030 - 2. Increase result.
030 - 3. No effect.
030 - 4. Centrifuge the mixture and use the supernatant.
030 - 5. Dilute the mixture 1:1 with distilled water, read, then multiply the results by 2.
030 - 6. Stored in an opaque container out of direct sunlight.

030 - 7. a. Line voltage fluctuations in the spectrophotometer.
b. Loose electrical connection.
c. Faulty wavelength calibration.
030 - 8. ±0.5 grams percent.
030 - 9. Errors due to incorrect blood volume and pipetting errors in general.
030 - 10. (a) Incorrect pipette calibration, (b) dirty cuvettes or dirty pipettes, (c) instrument errors, (d) incorrect dilutions, (e) unmatched cuvettes (flow through instruments excepted), (f) faded blank, or (g) deteriorated Drabkin's solution.
030 - 11. They provide speed, accuracy, and high precision in determinations.
030 - 12. Oxyhemoglobin, reduced hemoglobin, and carboxyhemoglobin.
030 - 13. The technician can pipette with increased reproducibility.

031 - 1. F. They are not.
031 - 2. F. Carbon monoxide.
031 - 3. T.
031 - 4. F. 2 to 10 percent.
031 - 5. F. The ferrour ion has been oxidized to the ferric state and is incapable of combining with or transporting the oxygen molecule which is replaced by an hydroxyl radical.
031 - 6. F. 1 to 2 percent.
031 - 7. F. It is not normally found in blood.
031 - 8. T.
031 - 9. T.

032 - 1. F. Results will be unaffected; bubbles indicate poor technique.
032 - 2. T.
032 - 3. F. Vertical position or centrifuged again.
032 - 4. F. ±2 percent.
032 - 5. F. Elevated.
032 - 6. T.
032 - 7. T.
032 - 8. F. Falsely low readings due to shrinkage of red blood cells.
032 - 9. F. Erroneously low results.
032 - 10. T.

033 - 1. T.
033 - 2. F. Greater than 30.
033 - 3. F. Percentage of the mean.
033 - 4. T.
033 - 5. T.
033 - 6. F. Entered in column D; then the squared differences entered in column E. (Fig. 2-12.)
033 - 7. F. ±2 SD.
033 - 8. F. ±1 SD = .12; ±2 SD = .24; ±3 SD = .36; mean = 14.2. 14.2 = .36 = 14.56; 14.56 is closer to +3 SD than +2 SD.

034 - 1. F. Control should be allowed to warm up to room temperature.
034 - 2. T.
034 - 3. F. ±2.
034 - 4. T.
034 - 5. F. Flaw in the test—deteriorated reagents, improper pH, inaccurate temperature, or defective instrument.
034 - 6. F. Enter cause, if determined, and corrective action taken for each procedure. This data may prove useful in troubleshooting future analytic problems.
034 - 7. T.

CHAPTER 3

035 - 1. d.
035 - 2. d.
035 - 3. g.
035 - 4. g.
035 - 5. c.
035 - 6. e.
035 - 7. c.
035 - 8. e.
035 - 9. b.
035 - 10. b.
035 - 11. f.
035 - 12. a.

036 - 1. i.
036 - 2. l.
036 - 3. l.
036 - 4. m.
036 - 5. k.
036 - 6. k.
036 - 7. n.
036 - 8. j.
036 - 9. p.
036 - 10. q.
036 - 11. q.
036 - 12. o.
036 - 13. a.
036 - 14. d.
036 - 15. b.
036 - 16. b.
036 - 17. c.
036 - 18. c.
036 - 19. e.
036 - 20. f.
036 - 21. h.
036 - 22. g.

037 - 1. An increase in blood cell concentration resulting from plasma loss, as in burns, and dehydration from excessive vomiting, diarrhea, sweating, or stress. Most common.
037 - 2. It is frequently a response to hypoxia. It may result from prolonged exposure to high altitude, anoxia due to congenital heart disease, or chronic exposure to chemicals which inactivate the oxygen-carrying potential of hemoglobin, for example, nitrate and carbon monoxide.
037 - 3. Condition of unknown cause.
037 - 4. Widespread stimulation of the entire bone marrow, with overproduction involving granulocytes and platelets as well as erythrocytes.

038 - 1. An alteration in the quality of RBCs may also indicate anemia.
038 - 2. Normochromic—normal amount; hypochromic—less than normal amount.
038 - 3. Diets, intrinsic, vitamin B_{12}, folic acid, gastric absorption, liver storage.
038 - 4. An inherited abnormality of hemoglobin.
038 - 5. Sickle cell anemia and thallasemia.
038 - 6. Endocrine disorders, infection, poisons, or radiation.
038 - 7. Poor diet, malabsorption, or poor iron utilization.
038 - 8. Free hemoglobin impairs the oxygen-carrying capacity of red blood cells.
038 - 9. Congenital hemolytic anemia.
038 - 10. Bacteria or parasitic infections, such as malaria; chemicals or drugs, such as arsenic, lead, and sulfonamides; or extensive burns.
038 - 11. Antibodies weaken the cellular membrane.

039 - 1. g.
039 - 2. c.
039 - 3. g.
039 - 4. c.
039 - 5. f.
039 - 6. f.
039 - 7. b.
039 - 8. e.
039 - 9. a.
039 - 10. a.
039 - 11. d.

040 - 1. T.
040 - 2. F. Will show.
040 - 3. T.
040 - 4. F. It is difficult to make any distinction. Only "positive" or "negative" is reported.
040 - 5. F. 20 µl (o.2 ml).
040 - 6. F. Solution will be clear, and the lines on the reader scale will be visible through the solution.
040 - 7. F. Hemoglobin electrophoresis should be performed if the dithionite tube test is positive.
040 - 8. F. Dithionite tube test and urea-dithionite test.
040 - 9. F. The use of 12-x 75-mm test bubes may result in a flase negative.
040 - 10. T.
040 - 11. T.
040 - 12. T.
040 - 13. T.
040 - 14. F. Hemoglobin Bart, Hgb C-Harlem, and the presence of certain abnormal proteins.

041 - 1. It is used in a general sense to detect, confirm, and/or follow the course of a disease process.
041 - 2. Rouleaux formation, mass agglutination of erythrocytes, and severe macrocytosis.
041 - 3. Fibrinogen and globulin.
041 - 4. They are unable to agglutinate and form a mass due to the alteration of their shape.
041 - 5. The erythrocyte concentration in blood is decreased and settling out is easier and more rapid.
041 - 6. Fibrinogen, alpha-1 globulin, and alpha-2 globulin.
041 - 7. ESR is increased.
041 - 8. Adults over 60.
041 - 9. It will denote the presence of disease, but not the severity.

042 - 1. When the dilution factor is introduced, the values tend to be higher.
042 - 2. None; 5 percent with double oxalate.
042 - 3. 22° to 27° C.
042 - 4. Decreases the sedimentation rate.
042 - 5. 30 percent.

043 - 1. (1) Mean corpuscular volume (MCV), (2) mean corpuscular hemoglobin (MCH), and (3) mean corpuscular hemoglobin concentration (MCHC).
043 - 2. a. Red blood cell count.
b. Hematocrit.
c. Hemoglobin.
043 - 3. $\frac{42 \times 10}{6} = 70$ fl.
043 - 4. Microcytic.
043 - 5. $\frac{12 \times 10}{4} = 30$ picograms.
043 - 6. Mean corpuscular hemoglobin concentration (MCHC).
043 - 7. $\frac{14 \times 100}{45} = 31$ g/dl.

044 - 1. The osmotic pressure of the fluid.
044 - 2. Crenation occurs.
044 - 3. The cell becomes spheroid along with crenations; the crenated spheroid loses its crenation and hemolyzes; and a ghost cell remains.
044 - 4. Congenital spherocytic anemia, hemolytic disease of the newborn, chemical poisoning, liver cases.
044 - 5. The osmotic pressures of the cell and the saline are balanced.
044 - 6. The blood specimen may be collected aseptically in a sterile container with glass beads and incubated 24 hours at 37° C before running the test.

CHAPTER 4

045 - 1. (a) Cell size decreases; (b) nuclear chromatin becomes more clumped (compact); (c) straining reaction of cytoplasm changes; (d) younger cells are basophilic.
045 - 2. Azurophilic or nonspecific granules.
045 - 3. They originate in the bone marrow. A secondary potential is the reticuloendothelial system, which includes the spleen and liver.
045 - 4. (a) Myeloblast; (b) promyelocyte; (c) myelocytr; (d) metamyelocyte; (e) band; (f) segmented cell.

046 - 1. c.
046 - 2. c.
046 - 3. a.
046 - 4. c.
046 - 5. a.
046 - 6. a.
046 - 7. d.
046 - 8. a.
046 - 9. d.
046 - 10. b.
046 - 11. d.
046 - 12. d.
046 - 13. b, d.

047 - 1. f.
047 - 2. h.
047 - 3. c.
047 - 4. f.
047 - 5. f.
047 - 6. b, e.
047 - 7. b, e.
047 - 8. i.
047 - 9. a, d.
047 - 10. d, j.
047 - 11. b, e.
047 - 12. c, j.

048 - 1. T.
048 - 2. F. Megakaryocytes.
048 - 3. F. Megakaryocytes.
048 - 4. T.
048 - 5. F. Larger, ranging up to 50 micrometers in diameter.
048 - 6. T.
048 - 7. T.
048 - 8. T.

049 - 1. The lymphatic system.
049 - 2. Lymphocyte.
049 - 3. Prolymphocyte.
049 - 4. The nuclear chromatin is finer than the lymphocyte but not as delicate as in the myeloblast.

050 - 1. There are usually no granules in the cytoplasm of the monoblast, but there are very fine, lilac staining granules (azurophilic dust) in the cytoplasm of the promonocyte.
050 - 2. The nucleus of the monoblast usually shows one or two nucleoli, and the promonocyte shows zero to one nucleoli.
050 - 3. Slow, ameboid movement. This may be seen on the stained slide with single or multiple pseudopods.
050 - 4. Usually folded, but it may be round, kidney shaped, or deeply indented.
050 - 5. The very fine diffuse chromatin strands with abundant parachromatin.
050 - 6. The cytoplasm of a monocyte is opaque gray-blue without the clear perinuclear zone described for most lymphocytes. There is a large amount of cytoplasm in relation to the nucleus, in contrast to the lymphocyte.
050 - 7. Metamyelocyte.
050 - 8. The brainlike convolutions of the nucleus and the dull gray-blue color of the cytoplasm.

051 - 1. Compression is due to the pressure of cells upon each other, and crushing is due to mechanical pressure which ruptures cells when the smear is made.
051 - 2. Compressed cells appear smaller than normal with darker staining cytoplasm.
051 - 3. They are easily crushed.
051 - 4. Spindle forms; smudge or basket cells.

052 - 1. g.
052 - 2. f.
052 - 3. c.
052 - 4. h, k.
052 - 5. i.
052 - 6. i.
052 - 7. g, m, j.
052 - 8. c.
052 - 9. j.
052 - 10. a, j.
052 - 11. e. Table 4-1.
052 - 12. m.
052 - 13. d. Table 4-1.
052 - 14. b. Table 4-1.
052 - 15. n.
052 - 16. h, m.

053 - 1. May be involvement of the whole body, especially lymphatic tissues, and signs varying with the organs and tissues involved.
053 - 2. Monospot test.
053 - 3. There is no correlation.
053 - 4. Vacuolated cytoplasm, deeply basophilic cytoplasm, and nucleoli in the nucleus.
053 - 5. Any one of these three morphological characteristics, or any combination, is indicative of an atypical lymphocyte.
053 - 6. Serological tests, such as the monospot test or differential absorption test.
053 - 7. Small, mature lymphs.
053 - 8. Negative.

054 - 1. Leukemia is an abnormal, uncontrolled proliferation of the white cell producing cells in the bone-forming organs and peripheral blood.
054 - 2. A very high white count consisting of cells of the granulocytic series in various stages of maturation and usually many mature forms.
054 - 3. The leukocyte alkaline phosphatase test, which is low in myelogenous leukemia.
054 - 4. It is associated with chromosomal abnormality on the 21st chromosome.
054 - 5. Peroxidases are normally present in cells of the granulocytic series and negative in those of the lymphocytic series and basophils.
054 - 6. Large numbers of mature lymphocytes. The bone marrow, spleen, and lymph nodes are markedly affected.
054 - 7. They usually have a rapid course and are characterized by numerous blast cells in the peripheral blood.
054 - 8. Acute lymphocytic leukemia.
054 - 9. Thin, eosinophilic, rod-shaped structures observed in the cytoplasm of some granulocytes in acute myelogenous leukemia.

055 - 1. An abnormal protein is present, resulting in degeneration of nuclear material, which then becomes phagocytized by other cells.
055 - 2. Free masses of lysed nuclear material with or without polymorphonuclear leukocytes clustered about them (rosette formation).
055 - 3. A true L.E. cell is a neutrophilic granulocyte or phagocyte with a homogenous cytoplasmic inclusion which has no chromatin pattern. A tart cell is usually a monocyte which has ingested another cell or the nucleus of another cell with a recognizable pattern and nuclear membrane.
055 - 4. Negative.

CHAPTER 5

056 - 1. *Hemostasis* is a complex process in which several factors work together or in sequence to stop the flow of blood from an injured blood vessel.
056 - 2. The *vascular mechanism* involves the veins, arteries, and capillaries themselves. They aid in stopping the flow of blood by contracting the retracting to close off the vessel.
056 - 3. The intrinsic thromboplastin mechanism involves coagulation of the blood itself. This is a highly complicated mechanism that requires several stages for completion. These stages are the generation of thromboplastin, the generation of thrombin, and the production of fibrin.
056 - 4. The extrinsic thromboplastin mechanism involves the surrounding tissue, such as skin, muscle, and subcutaneous tissues. These tissues help stop the flow of blood by contracting or by virtue of their weight and thickness.

057 - 1. F. Liquid blood.
057 - 2. T.
057 - 3. F. Platelets.
057 - 4. T.
057 - 5. F. Prothrombin activating component; platelet factor 3.

058 - 1. e.
058 - 2. e.
058 - 3. b, c, g, i.
058 - 4. i.
058 - 5. b, c, g, i.
058 - 6. d.
058 - 7. d.
058 - 8. l.
058 - 9. h.
058 - 10. c.
058 - 11. c.
058 - 12. k.
058 - 13. k.
058 - 14. g.
058 - 15. g.
058 - 16. g.
058 - 17. b.
058 - 18. b, c, g, i.
058 - 19. j.
058 - 20. f.
058 - 21. a.
058 - 22. a.

059 - 1. Prothrombin, thromboplastin, thrombin, and fibrinogen.
059 - 2. (1) The formation of plasma thromboplastin; (2) the conversion of prothrombin to thrombin; (3) the formation of fibrin from fibrinogen.
059 - 3. Vascular injury and contact with collagen.
059 - 4. Factor VIII, calcium ions, and phospholipids from platelets (PF3).
059 - 5. Tissue thromboplastin (factor III, calcium ions, and factor VII) reacts with factor X.
059 - 6. Plasma thromboplastin, with calcium ions, converts prothrombin to thrombin.
059 - 7. Thrombin.
059 - 8. Release of fibrinopeptide A from the alpha chain first and then the release of fibrinopeptide B.

060 - 1. Antithromboplastin which binds factor VIII, and probably factor IX, making them unavailable for the generation of thromboplastic activity.
060 - 2. Inhibitors specific for factor VIII and factor IX.
060 - 3. It combines with the heparin cofactor, an alpha globulin, to form a potent antithrombin.
060 - 4. The coagulation time and partial thromboplastin time.
060 - 5. It acts as an antivitamin K and will decrease the production of normal factor II, factor VII, factor X, and factor IX.
060 - 6. The prothrombin time.

061 - 1. T.
061 - 2. F. Except factor VII and platelets.
061 - 3. F. A more specific testing procedure is required.
061 - 4. T.
061 - 5. F. Prothrombin consumption and whole blood clotting time.

*U.S. GOVERNMENT PRINTING OFFICE: 1981 546-578

061 - 6. F. Unconverted prothrombin.
061 - 7. T.
061 - 8. F. The prothrombin time.
061 - 9. T.
061 - 10. F. Coumadin anticoagulant therapy.
061 - 11. T.
061 - 12. T.

062 - 1. Keeps the vascular system free of deposited fibrin or fibrin clots.
062 - 2. The prevention of thrombosis.
062 - 3. Plasmin or fibrinolysin.
062 - 4. An inactive precursor, plasminogen, or profibrinolysin.
062 - 5. (1) A soluble-phase, free plasminogen that circulates in the plasma with other plasma protein; and (2) a gel phase, the plasminogen that is absorbed to fibrinogen.
062 - 6. Because antiplasmin is capable of effectively neutralizing low concentrations of plasmin.
062 - 7. Generally when fibrinolysis is increased, coagulation is increased.

063 - 1. Hemophilia A involves factor VIII deficiency, and hemophilia B involves factor IX deficiency.
063 - 2. Both diseases are inherited as sex-linked, recessive characteristics.
063 - 3. Hemophilia is inherited as a trait carried on the X-chromosome. Since males have only one X-chromosome, they would be more likely to show the expressed condition.
063 - 4. Hemophilia B.
063 - 5. There is greater stability of factor IX in stored blood.
063 - 6. The genes are carried on the autosomes, but they are not inherited as sex-linked characteristics.

064 - 1. (1) Liver diseases, (2) hyperfunction of the spleen, (3) hypersensitivity to drugs with demonstrable immunologic reaction, (4) the septicemias, (5) bone marrow hypoplasia, (6) mass transfusions, and (7) circulating anticoagulants.
064 - 2. (1) Activated partial thromboplastin time, (2) platelet count, (3) bleeding time, (4) prothrombin time, and (5) clotting time.

065 - 1. A crude measure of capillary fragility.
065 - 2. (a) Qualitative and quantitative platelet abnormalities, (b) vitamin C deficiency, and (c) increased bleeding time.

066 - 1. F. A small *standardized* wound.
066 - 2. F. The elasticity of the skin and capillary vessels, the efficiency of tissue fluids, and the mechanical and chemical action of the platelets.
066 - 3. F. Values may be difficult to reproduce.
066 - 4. F. You should not stimulate the circulation by rough handling.
066 - 5. F. The forearm.
066 - 6. T.
066 - 7. F. Squeezing must not be done.
066 - 8. T.
066 - 9. F. Too deep a wound prolongs the bleeding time, while a shallow wound shortens it.
066 - 10. F. Ivy bleeding.
066 - 11. F. Depends upon the extravascular and vascular factors and to a lesser degree on coagulation factors.
066 - 12. T.
066 - 13. T.

067 - 1. A rough measure of all intrinsic clotting factors in the absence of tissue factors.
067 - 2. Plasma thromboplastin is produced; then a few seconds are required to convert prothrombin to thrombin and fibrinogen to fibrin.
067 - 3. Stage I, since plasma thromboplastin is generated in this stage.
067 - 4. (a) Moderately severe hemophilia, factor VIII deficiency; (b) afibrinogenemia; and (c) severe fibrinolytic conditions.
067 - 5. Thromboembolic disorders.
067 - 6. The prothrombin time.
067 - 7. (a) Temperature variations, (b) test tube size, (c) surface of tube, (d) volume of the blood in the tube, and (e) frequency and manner of tilting the tubes.
067 - 8. (a) Rough handling, (b) presence of tissue fluids, (c) frequency tilting, (d) unclean tubes, and (e) quantities less than 1 ml of blood.
067 - 9. (a) Extreme increases in temperature, (b) variation in pH, (c) performance of the test at room temperature, (d) quantities greater than 1 ml of blood, and (e) bubbles entering the syringe when sample is taken.
067 - 10. Normal. Only a small number of thrombocytes need be present for normal coagulation to take place.
067 - 11. Clot retraction.
067 - 12. To observe for clot lysis and/or erythrocyte fallout and complete clot retraction.

068 - 1. F. A function of intact platelets, the fibrinogen content of the plasma, and the ratio of plasma volume to red cell mass.
068 - 2. T.
068 - 3. F. Serum expressed.
068 - 4. F. Platelet deficiency.
068 - 5. F. Thrombocytopenia, qualitative platelet deficiency, and in cases of an increase in red cell mass.
068 - 6. T.
068 - 7. T.
068 - 8. F. Plasmin.
068 - 9. F. Plasmin acts to dissolve the clot.
068 - 10. F. Mistures of patient's plasma, normal plasma, and thrombin.
068 - 11. F. Negative.

069 - 1. F. Results are significantly lower and less satisfactory.
069 - 2. F. Brilliant cresyl blue.
069 - 3. F. Sodium citrate.
069 - 4. T.
069 - 5. F. 16 to 25 percent.
069 - 6. T.
069 - 7. F. Ammonium oxalate.
069 - 8. T.
069 - 9. F. 10 percent.
069 - 10. F. More accurate.
069 - 11. F. It does overcome this difficulty.
069 - 12. T.
069 - 13. T.

070 - 1. (a) sedimentation, providing separation of RBCs in 10 to 50 minutes; (b) closely controlled centrifugation at 300 g for 5 minutes; (c) centrifugation of tube held in vertical position at 40 g for 25 minutes.
070 - 2. (a) Coincidence, (b) dilution, (c) hematocrit.
070 - 3. Platelets entering the aperture at the same time as the red cells will not be detected.
070 - 4. The count will be falsely elevated.
070 - 5. (a) The red cell count, (b) the number of platelets noted per 1000 RBCs.

071 - 1. Mainly in the diagnosis and treatment of the hemorrhagic diseases, surgical hemorrhage, or obstetrical bleeding.
971 - 2. To the Simplastin solution of calcium and thromboplastin.
071 - 3. The clot will be partially or totally dissolved, and erroneous results will be reported.
071 - 4. Latex antihuman fibrinogen reagent.
071 - 5. Ammonium sulfate.
071 - 6. Fresh every 2 weeks.

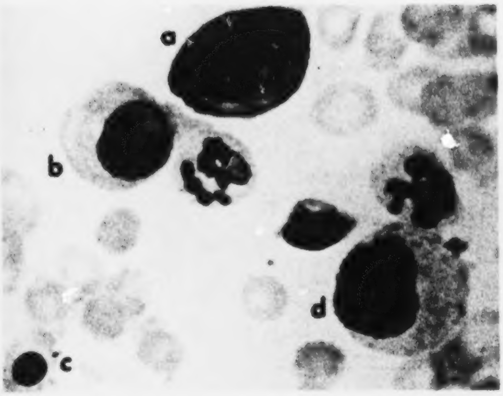
072 - 1. Calcium.
072 - 2. The concentration of prothrombin and accessory factors.
072 - 3. Extrinsic.
072 - 4. Factor V is labile in oxalated blood.

072 - 5. Refrigerate until ready for use.
072 - 6. 10 minutes.
072 - 7. Factor V.
072 - 8. Factor I, II, V, VII, or X.
072 - 9. Barbiturates, digitalis, diuretics, vitamin K, and oral contraceptives.
072 - 10. ±0.5 seconds.
072 - 11. It allows to a great extent for standardization and uniformity independent of the time of day, the day of the week, or technician's skill.

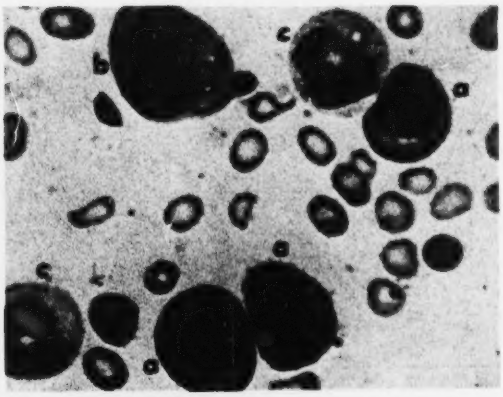
073 - 1. F. Prothrombin to thrombin.
073 - 2. T.
073 - 3. F. Factors VIII and IX, and platelet factor 3 or adequacy of platelets.
073 - 4. F. Tissue thromboplastin, calcium, factor V, and fibrinogen.
073 - 5. F. Factors VII, IX, X, XI, and XII.
073 - 6. F. May *not*.
073 - 7. T.
073 - 8. F. Must not be held for more than 2 hours at 4° C.
073 - 9. T.
073 - 10. T.
073 - 11. T.

074 - 1. VII, XIII, platelet factor 3.
074 - 2. Calcium, platelets.
074 - 3. Activator.
074 - 4. Calcium chloride, kaolin suspension, control plasma, partial thromboplastin.
074 - 5. Glassware surface, time in contact with the glass, type of syringe used.
074 - 6. Kaolin.
074 - 7. Platelin or activated cephaloplastin, patient's plasma, calcium chloride.
074 - 8. 45.
074 - 9. Buffy coat.
074 - 10. Lupus erythematosus, heparin, fibrin degradation products (fibrinolysins, antifactor VIII antibody).
074 - 11. APTT.
074 - 12. The anticoagulant used to obtain the blood, the type of equipment used, the individual running the test.

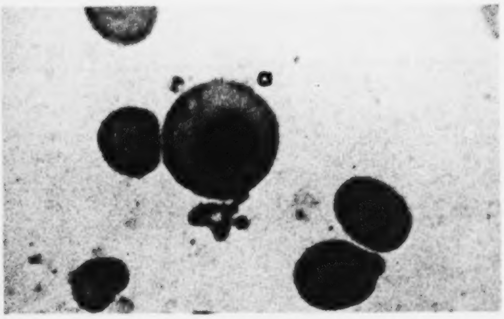
075 - 1. F. Plasma or intrinsic thromboplastin.
075 - 2. F. I and II.
075 - 3. T.
075 - 4. F. Not able to distinguish between the two.
075 - 5. F. Able to distinguish between the two.
075 - 6. T.
075 - 7. F. Factors I, II, V, VII, and X.
075 - 8. F. Factors V, VIII, XI, and XII.
075 - 9. F. Partial thromboplastin serves as a platelet-like substance.
075 - 10. T.
075 - 11. T.
075 - 12. T.



a. Rubriblast
b. Prorubricyte (Pernicious Anemia)
c. Metarubricyte
d. Myelocyte

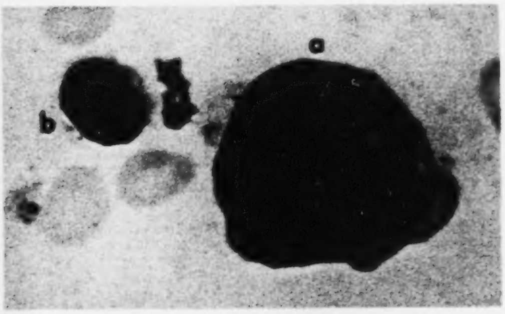


a. Prorubricytes
b. Metamyelocyte
c. Bands

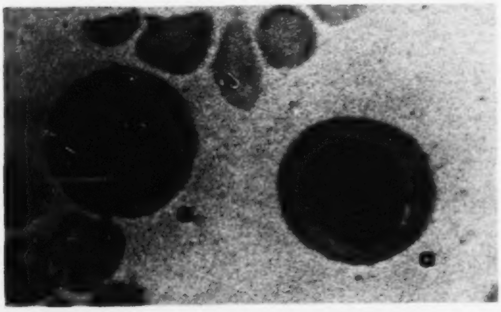


a. Metarubricyte (Pernicious Anemia)

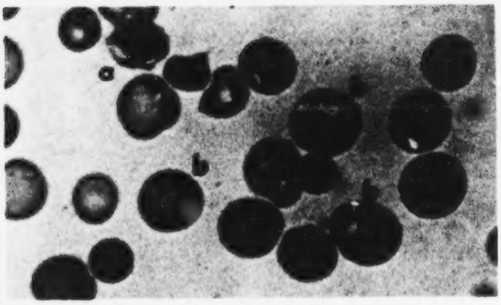
a. Metarubricyte
b. Rubriblast
c. Prorubricyte



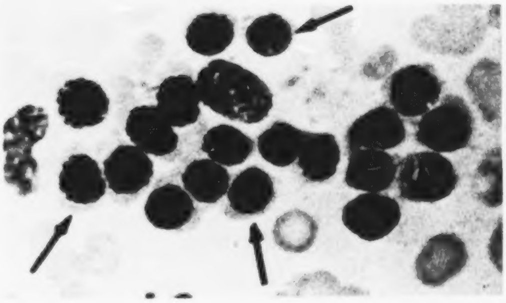
a. Prorubricyte (Megaloblastic)
b. Lymphocyte



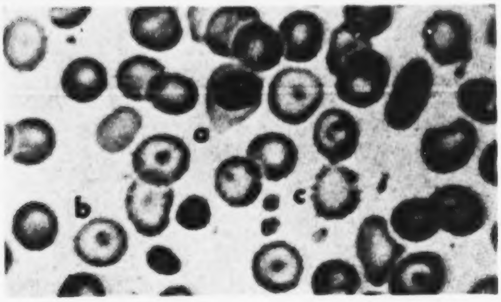
a. Rubricytes (Pernicious Anemia)



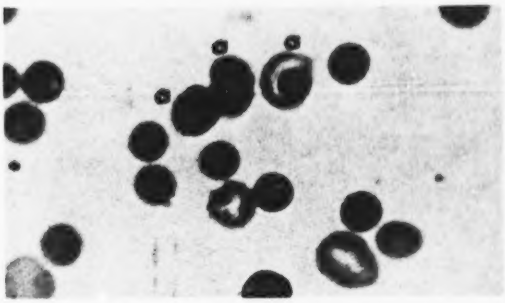
a. Metarubricyte
b. Howell-Jolly Bodies
Hypochromic Macrocytic Erythrocytes



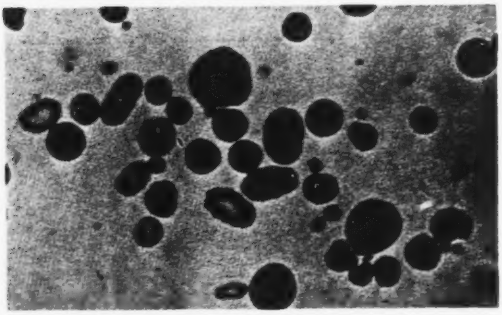
Rubricytes



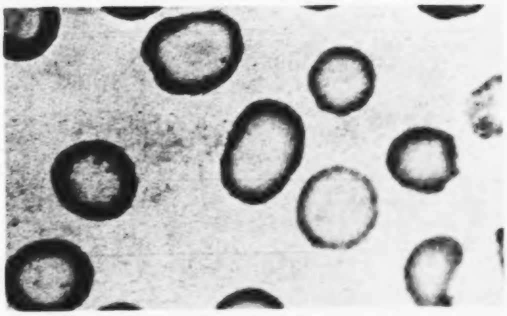
a. Metarubricyte
b. Target Cell
c. Crenated RBC



a. Metarubricytes
Anisocytosis
Polychromasia
Spherocytosis

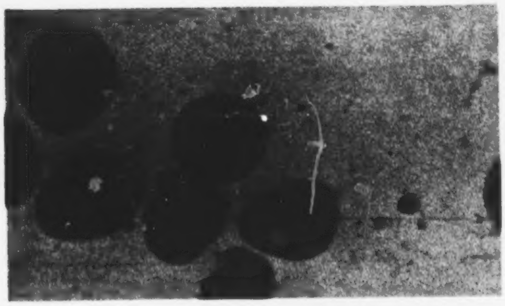


Hereditary Spherocytosis

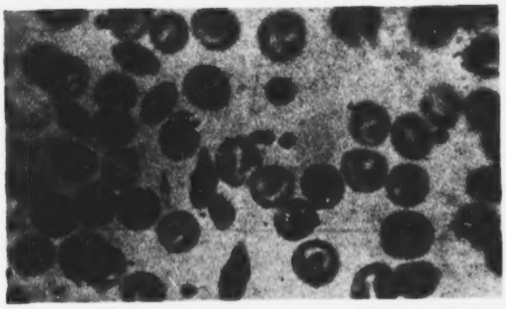


Hypochromic Macrocytic Erythrocytes

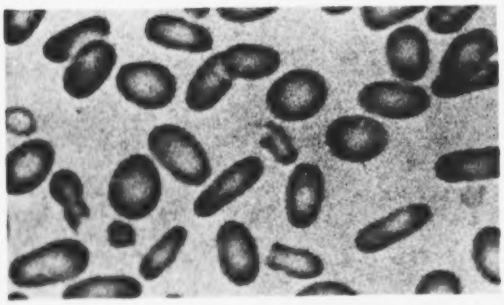
Foldout 1. RED BLOOD CELL MATURATION



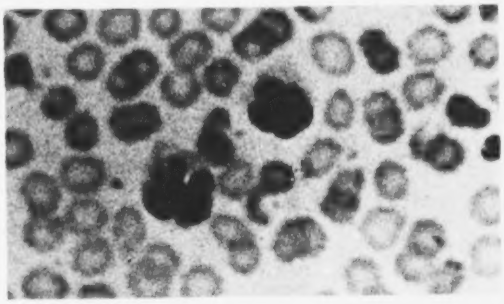
a. Basophilic Stippled Erythrocyte



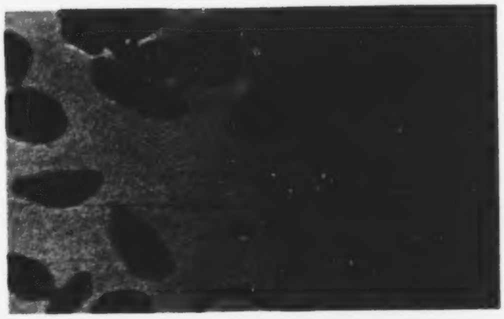
Marked Poikilocytosis
Anisocytosis and
Target Cells



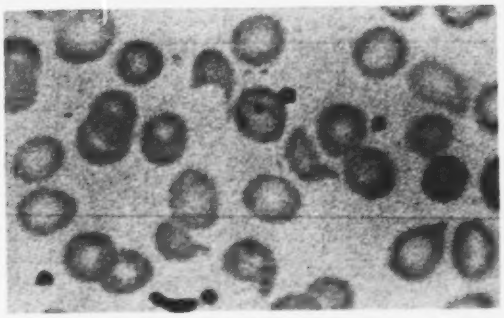
Elliptocytes (Oval Erythrocytes)



Crenated RBC
Burr Cells
Acanthocytes
2 Leukocytes



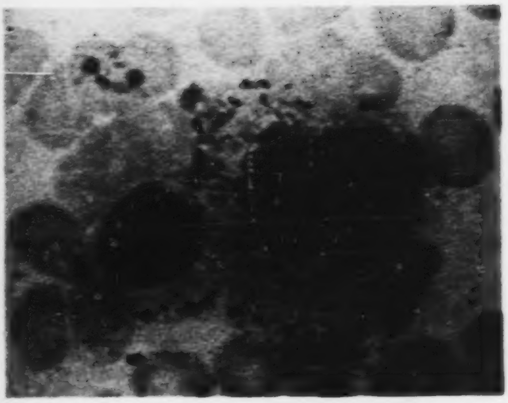
Poikilocytosis: Sickle Cells



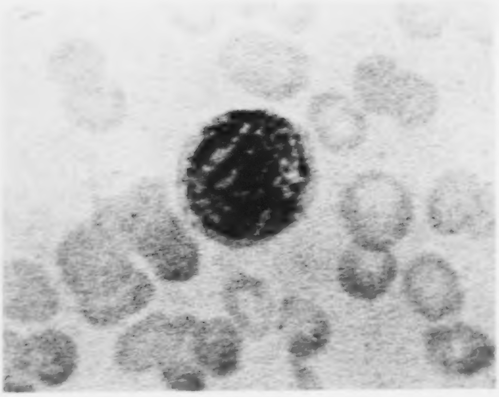
a. Cabot Ring

AND MORPHOLOGY

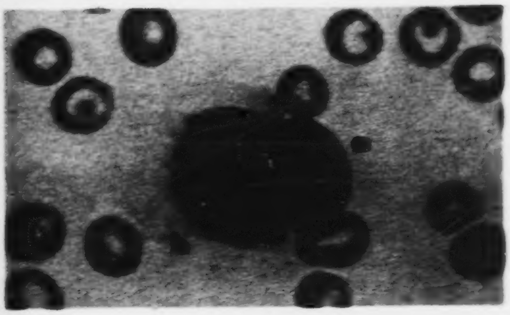
Illustrations Courtesy of Armed Forces Institute of Pathology



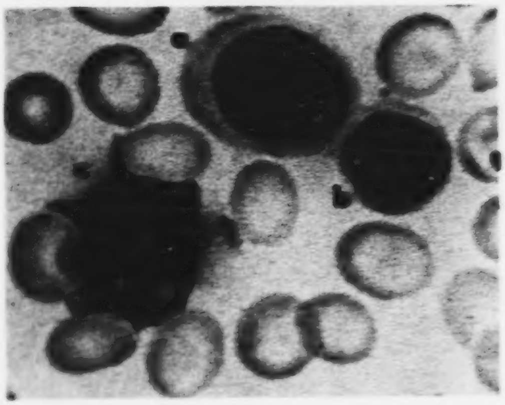
a. Stem (Ferrata) Cell
b. Myelocyte



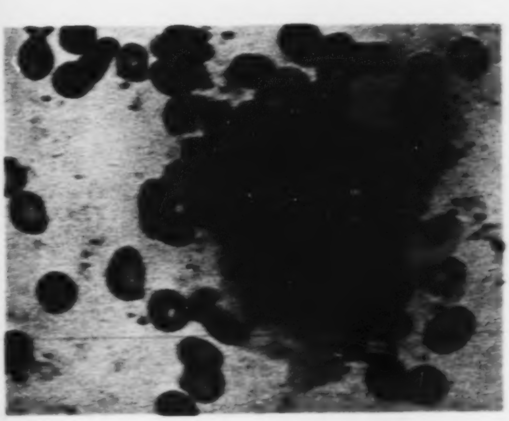
Myeloblast



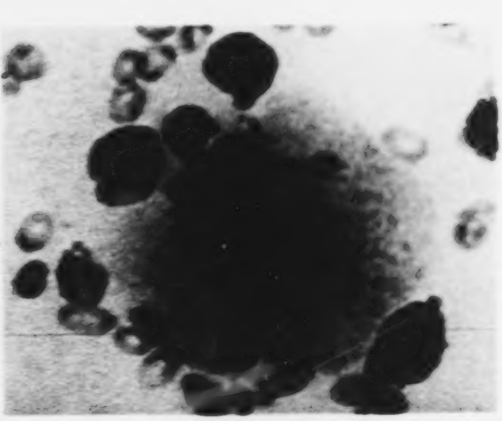
a. Monoblast
b. Stem (Ferrata) Cell



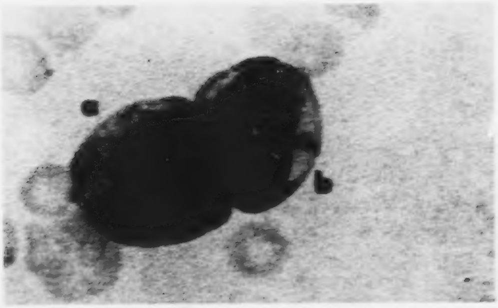
a. Lymphoblast
b. Lymphocyte
c. Smudge Cell



Megakaryoblast: Bone Marrow



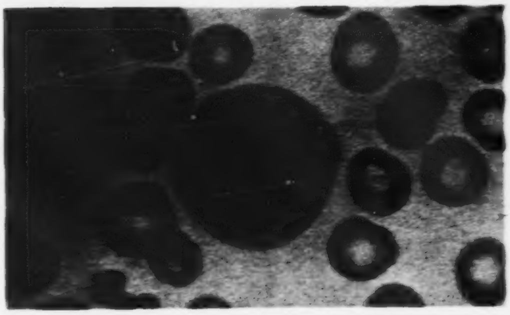
Megakaryocyte: Mature



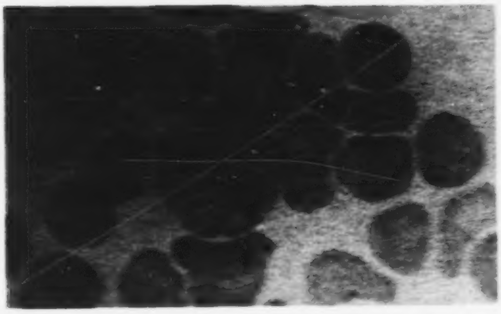
a. Promyelocyte
b. Promyelocyte with Auer Body

Atypical Lymphocytes: Infectious Mononucleosis

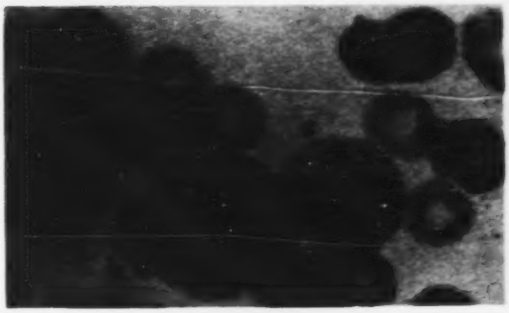
L. E. Cells



Neutrophil: Hypersegmented

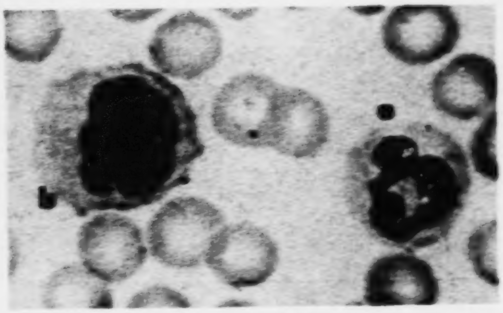


Band Neutrophil: Toxic Granulation

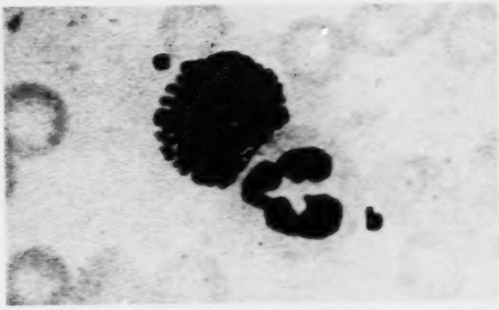


a. Lymphocyte
b. Monocyte
c. Neutrophil Segmented

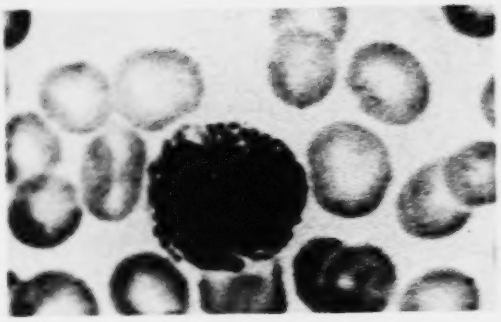
Foldout 2. LEUKOCYTE MATURATION



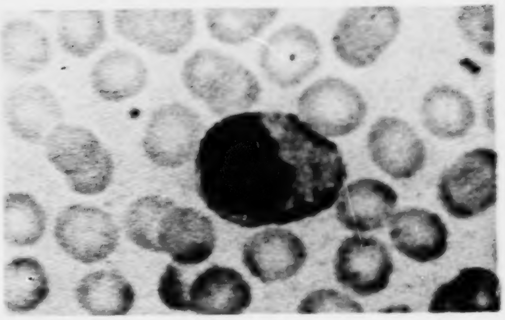
a. Neutrophil (Late Band)
b. Monocyte



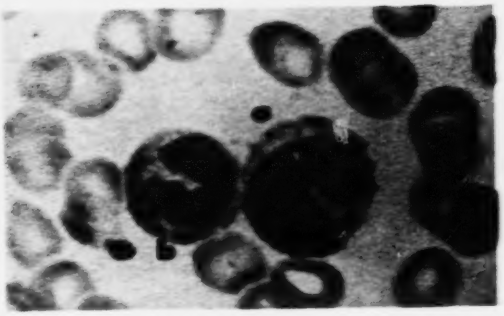
a. Basophil
b. Neutrophil: Segmented



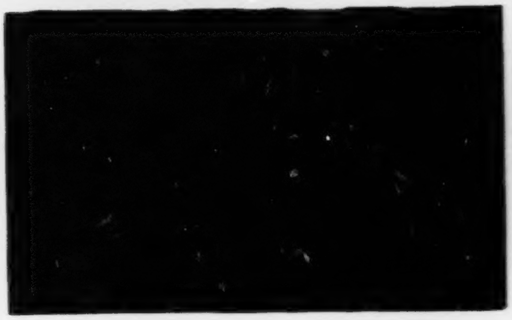
Eosinophil



Monocyte



a. Metamyelocyte
b. Band Neutrophil



Lymphocyte: Azurophilic Granulation

AND MORPHOLOGY

Illustrations Courtesy of Armed Forces Institute of Pathology

STOP-

1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.

2. USE NUMBER 2 PENCIL ONLY.

90413 01 22

EXTENSION COURSE INSTITUTE
VOLUME REVIEW EXERCISE
HEMATOLOGY

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that item numbers on answer sheet are sequential in each column.
3. Use a medium sharp #2 black lead pencil for marking answer sheet.
4. Write the correct answer in the margin at the left of the item. (When you review for the course examination, you can cover your answers with a strip of paper and then check your review answers against your original choices.) After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if a all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DONT'S:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than a #2 black lead pencil.

NOTE: NUMBERED LEARNING OBJECTIVE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Learning Objective Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Learning Objectives* indicated by these *Numbers*. The VRE results will be sent to you on a postcard which will list the *actual* VRE *items you missed*. Go to the VRE booklet and locate the *Learning Objective Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (001) Under normal conditions, where are an adult's red blood cells produced?

   a. The yellow marrow of the long bones.
   b. Both the spleen and the red marrow.
   c. The shaft of the long bones.
   d. The red marrow of the spongy bone.

2. (001) During the third and fourth months prior to birth and until several weeks after, leukocytes are produced by the

   a. lymph nodes and thymus.
   b. liver.
   c. lymph nodes and spleen.
   d. spleen.

3. (002) Erythrocytes are normally disposed of by the reticuloendothelial system, and in particular by the

   a. bone marrow.
   b. lymph nodes.
   c. liver.
   d. spleen.

4. (003) The exchange of oxygen and carbon dioxide between the alveoli and the blood is best described by which of the following functions?

   a. Breathing.
   b. Internal respiration.
   c. External respiration.
   d. Expiration.

5. (003) If air in the alveoli at sea level contains water vapor at a pressure of 47 mm Hg, the resultant effective gas pressure in the alveolus is what value?

   a. 713 mm Hg.
   b. 47 mm Hg.
   c. 807 mm Hg.
   d. Cannot be determined from data given.

6. (004) The exchange of gas between the blood and the tissue cell defines

   a. tissue exchange.
   b. osmosis.
   c. internal respiration.
   d. external respiration.

7. (004) The so called normal form of hemoglobin is designated by what letter?

   a. C.
   b. A.
   c. S.
   d. F.

8. (005) The hemoglobin molecule "carries" oxygen in what manner?

   a. Hemoglobin oxidizes to oxyhemoglobin; oxyhemoglobin reduces to the ferric state.
   b. Hemoglobin oxidizes to carboxyhemoglobin; carboxyhemoglobin reduces to the ferric state.
   c. Hemoglobin oxidizes to oxyhemoglobin; oxyhemoglobin reduces to the ferrous state.
   d. Hemoglobin oxidizes to carboxyhemoglobin; carboxyhemoglobin reduces to the ferrous state.

9. (006) Which leukocyte is most closely associated with allergic reactions?

   a. Basophil.
   b. Monocyte.
   c. Lymphocyte.
   d. Eosinophils.

10. (006) Which cells are thought to be directly connected with antibody production?

a. Lymphocytes.
b. Monocytes.
c. Neutrophils.
d. Basophils.

11. (007) Which of the following sequences is correct for blood flow through the pulmonary circulatory system?

a. Superior and inferior vena cava, right atrium, aorta, and left atrium.
b. Left ventricle, aorta, vena cava, right atrium.
c. Pulmonary artery, lungs, aorta, and right atrium.
d. Right ventricle, pulmonary artery, lungs, pulmonary vein, and left atrium.

12. (008) The systolic pressure in the normal adult falls within what range?

a. 30-60 mm Hg.
b. 60-90 mm Hg.
c. 90-140 mm Hg.
d. 120-150 mm Hg.

13. (009) Differences in cell concentrations occur when blood is obtained from the ear lobe, due to high concentrations of

a. Neutrophils.
b. Eosinophils.
c. Monocytes.
d. Lymphocytes.

14. (010) During a venipuncture, if it is difficult to enter the vein, or if a hematoma forms, you should

a. release the tourniquet; continue to probe with needle until the vein is found.
b. keep the tourniquet on the patient's arm for 20 minutes; then once more attempt to enter the vein.
c. release the tourniquet promptly, withdraw the needle, and apply pressure to the puncture site.
d. keep the tourniquet on for 20 minutes, withdraw the needle, and apply pressure to the puncture site.

15. (010) If a patient has intravenous solutions going into both arms, from what site may blood be obtained?

a. Blood should not be obtained from either arm.
b. It is acceptable to puncture the veins 3 or 4 inches below the site of the IV.
c. Blood should not be obtained from either arm; a femoral will yield the only valid results.
d. It is acceptable to obtain the blood from the IV tubing without invalidating test results.

16. (011) After a few minutes of mixing with the blood, which of the following anticoagulants is most likely to cause crenation of RBCs, vacuoles in the granulocytes, and bizarre forms of lymphocytes and monocytes?

a. Sodium citrate.
b. EDTA.
c. Heparin.
d. Ammonium and potassium oxalate.

17. (011) The anticoagulant of choice for coagulation studies is

a. sodium citrate.
b. sodium oxalate.
c. heparin.
d. EDTA.

18. (012) Blood is drawn to the 0.3 mark in an RBC pipette and diluted to the 101 mark. What is the dilution and in what case would such a dilution be made?

a. A dilution of 1:333; in anemia.
b. A dilution of 1:222; in polycythemia.
c. A dilution of 1:333; in polycythemia.
d. A dilution of 1:222; in anemia.

19. (012) Why is Gower's solution considered superior to Hayem's solution for manual red cell count?

a. Hemolysis is kept minimal and overcharging is avoided.
b. Hemolysis is avoided and it enhances cell preservation.
c. It prevents rouleaux formation and enhances platelet counting.
d. It prevents rouleaux formation and clumping of the red cells.

20. (013) An increase in the RBC count noted in severe burn cases, severe vomiting, or persistent vomiting during pregnancy is most likely due to

a. excitement.
b. posture.
c. anemia.
d. dehydration.

21. (014) What would be the WBC count if 48 cells were counted in a case of leukopenia, and a 1:10 dilution was used with a WBC pipette?

a. 1,100 WBC/mm^3.
b. 1,200 WBC/mm^3.
c. 1,300 WBC/mm^3.
d. 1,400 WBC/mm^3.

22. (015) The yellow coloration of CSF is commonly referred to as

a. spinalgia.
b. xerasia.
c. xanthochromia.
d. polychromasia.

23. (015) In performing a routine CSF count, 15 cells are counted (low count, less than 500). What is the reported cell count?

a. 14/mm^3.
b. 15/mm^3.
c. 16/mm^3.
d. 17/mm^3.

24. (016) Upon receipt of a specimen for semen analysis, which procedure is least likely to be used?

a. Set in the incubator at 37° C. until liquefies.
b. Record time received and the time of collection.
c. Observe for and record turbidity, viscosity; determine pH.
d. Check for motility under high dry power.

25. (016) Which of the following values represents the normal range for sperm count in number per ml?

a. 6 to 15 million.
b. 60 to 150 million.
c. 160 to 300 million.
d. 250 to 400 million.

26. (017) The higher the cell count, the greater the probability that more than one cell will enter the aperture at one time, represents

a. coincidence inpulse.
b. coincidence passage.
c. impedance passage.
d. impedance impulse.

27. (018) In the model FN Coulter Counter, what control is used to determine the level above which the pulses of the particles will be counted?

a. Attenuation control.
b. Control piece.
c. Threshold dial.
d. Aperture current control.

28. (019) When Zap-Isoton® is used as a lysing agent, how long will the leukocyte remain preserved before destruction occurs?

a. 10 minutes.
b. 20 minutes.
c. 30 minutes.
d. 40 minutes.

29. (019) For accurate results, what is the counting time of the FN Coulter Counter?

a. 8-11 seconds.
b. 12-15 seconds.
c. 16-19 seconds.
d. 20-24 seconds.

30. (019) In a counting cycle with the model FN Coulter Counter, flashes on the screen would most likely indicate

a. bacterial contamination.
b. innumerable bubbles in the sample.
c. plugged orifice or contaminating debris.
d. high background count.

31. (020) In the Coulter Counter Model S, which of the following parameters are calculated by the analyzer?

a. WBC, RBC, MCHC.
b. PCV, MCH, MCHC.
c. WBC, MCH, PCV.
d. WBC, RBC, PCV.

32. (020) When debris, dirt, or a malfunction is present in any of the apertures of the Coulter Counter Model S, two of the three counts are rejected and the three data rejection lights are on, what count will be recorded?

a. 0.0001.
b. 0.0.
c. 0.01.
d. 0.001.

33. (021) The following components are basic subsystems of the laser hematology counter except the

a. mechanical.
b. optical.
c. analyzer.
d. electronic.

34. (021) In the Hemac laser hematology counter, how are the hematocrit and indices determined?

a. The hematocrit is calculated and the MCV, MCH, and MCHC are measured.
b. The hematocrit and MCV are measured and the MCH and MCHC are calculated.
c. The hematocrit is measured and the MCV, MCH, and MCHC are calculated.
d. The hematocrit is calculated with the MCHC and the MCH and MCV are measured.

35. (021) When hemoglobin values exceed 27 g/dl with the laser hematology counter, what must be done with the sample?

a. Dilute and repeat.
b. Repeat and report result; dilution is not necessary.
c. Repeat and dilute; only if the value has changed.
d. Do not repeat, report value as within reportable range.

36. (022) When electronic counter results do not correspond with the clinical situation, what source of error should be a prime consideration?

a. Dilution inaccuracies.
b. Plugged orifice or debris.
c. Contaminating particles and false impulses.
d. Incorrect labeling of sample source and number.

37. (022) When using electronic cell counter, the total white cell count in leukemia patients should be checked against the slide because leukemia cells may

a. disintegrate causing a falsely low leukocyte count.
b. disintegrate causing a falsely elevated leukocyte count.
c. agglutinate causing a falsely low leukocyte count.
d. agglutinate causing a falsely elevated leukocyte count.

38. (023) In performing a differential smear obtained from a patient with myelogenous leukemia, what extreme leukocyte condition is most likely to be observed?

a. Monocytosis.
b. Neutrophilia.
c. Basophilia.
d. Lymphocytosis.

39. (024) All routine differential blood smears should be kept in the laboratory for

a. 3 weeks.
b. 1 month.
c. 1 week.
d. 3 months.

40. (024) If a delay of more than 3 to 4 hours is necessary, slide smears for differentials should be fixed in what solution?

a. 95 percent isopropanol.
b. 95 percent ethanol.
c. 95 percent methanol.
d. 95 percent formalin.

41. (025) Dyes used to stain living cells are named

a. polychromatic.
b. Panoptic.
c. Viable.
d. supra-vital.

42. (025) In the Wright staining process, most of the staining occurs at what stage(s)?

a. Buffering stage.
b. Fixing stage.
c. Fixing and staining stages combined.
d. Buffering and fixing stages combined.

43. (026) On a differential smear, when RBCs stain blue and WBCs stain dark, what is the most likely condition of the stain?

a. A good stain.
b. Acid or excessively acid.
c. Neutral with precipitated stain.
d. Excessive alkaline.

44. (027) In attempting to identify a leukocyte not readily identifiable, which of the following criteria is least important?

a. Cell size.
b. Features of the nucleus.
c. Features of the cytoplasm.
d. The shape of the cell.

45. (027) The most distinctive feature of an immature nucleus is the

a. presence, absence, and number of nucleoli in the nucleus.
b. presence, absence, and number of granules surrounding the nucleus.
c. presence, absence, type, and quantity of granules surrounding the nucleus.
d. presence and type of granules in the cytoplasm of the cell.

46. (028) Normally, the average platelets on a differential smear per 100 RBCs is

a. 0 to 2.
b. 4 to 8.
c. 10 to 12.
d. 14 to 16.

47. (028) If seven eosinophils were counted in 200 leukocytes, what would be the total number of eosinophils reported on the differential?

a. 3.
b. 3.5.
c. 4.
d. 7.

48. (029) If traces of copper are present in the diluting fluid, the result may be lowered in which of the following methods of hemoglobin determination?

a. Cyanmethemoglobin.
b. Oxyhemoglobin.
c. Acid hematin.
d. Direct matching.

49. (029) Which method measures all forms of hemoglobin except sulfhemoglobin?

a. Oxyhemoglobin.
b. Acid hematin.
c. Cyanmethemoglobin.
d. Direct matching.

50. (030) When cloudiness in the hemoglobin sample is a result of hemoglobin S and C, what correction must be made?

a. Dilute mixture 1:1 with distilled H_2O, read, multiply results by 2.

b. Dilute mixture 1:10 with distilled H_2O, read, multiply results by 2.

c. Do not dilute; read sample and divide results by 2.
d. Do not dilute; read sample and multiply results by 2.

51. (031) Which of the following abnormal hemoglobin pigments is not reversible?

a. Carboxyhemoglobin.
b. Sulfhemoglobin.
c. Methemoglobin.
d. Ferrihemoglobin.

52. (032) If two microhematocrits have been performed, the results should agree within

a. ±0.5 percent.
b. ±1.0 percent.
c. ±2.0 percent.
d. ±3.0 percent.

53. (033) Coefficient of variation is expressed by what formula?

a. $100 \times \frac{\text{standard deviation}}{\text{mean}}$.
b. Mean x standard deviation.
c. 100 x standard deviation.
d. $\sqrt{\frac{\Sigma\ (a-b)^2}{2N}}$.

54. (033) The mean standard deviation is most meaningful if calculated in what manner?

a. For each test.
b. Daily.
c. Monthly.
d. Weekly.

55. (034) In the hematology quality control procedures, when the first control analysis falls within the limit and the second control analysis is out of limit, which of the following causes is least suspected?

a. Deteriorated reagents.
b. Improper pH.
c. Defective instrumentation.
d. Basic flaw in the control.

56. (035) Which of the following cells is the immediate precursor of the mature erythrocyte?

a. Rubricyte.
b. Prorubricyte.
c. Reticulocyte.
d. Rubriblast.

57. (035) Nucleoli can only be found in which of the following cells of the rubricytic series?

a. Rubricyte.
b. Prorubricyte.
c. Metarubricyte.
d. Rubriblast.

58. (036) Which of the following abnormal structures in erythrocytes may be described as blue, threadlike structures found in the RBCs of patients with severe anemia?

a. Howell-Jolly bodies.
b. Cabot rings.
c. Basophilic stippling.
d. Heinz-Ehrlich bodies.

59. (036) What structures, described as small, round, refractile inclusions inside the erythrocytes, are visible only in unfixed smears?

a. Heinz-Ehrlich bodies.
b. Howell-Jolly bodies.
c. Siderocytes.
d. Acanthocytes.

60. (037) A physiological condition with widespread stimulation of the entire bone marrow, with overproduction involving granulocytes and platelets as well as erthrocytes, is descriptive of which of the following polycythemias?

a. Relative polycythemia.
b. Secondary polycythemia.
c. Stress polycythemia.
d. Polycythemia vera.

61. (038) A red blood cell, which contains 35 percent hemoglobin, would be described as what in terms of color content?

a. Polychromatophilic.
b. Hyperchromic.
c. Normochromic.
d. Hypochromic.

62. (039) Which of the following anemias shows macrocytosis, poikilocytosis, polychromatophilia, granulocytopenia, and granulocytic hypersegmentation in the peripheral blood?

a. Aplastic.
b. Pernicious.
c. Mediterranean.
d. Congenital hemolytic.

63. (040) Homozygous S infants, less than 4 months old, will show what result in the sickle cell test and why?

a. RBCs more resistant to sickling due to hemoglobin F.
b. RBCs less resistant to sickling due to hemoglobin F.
c. RBCs more resistant to sickling due to hemoglobin A.
d. RBCs less resistant to sickling due to hemoglobin C.

64. (040) In the dithionite tube test method for sickle cell, which result is considered positive if hemoglobin S is present?

a. Solution is clear; lines on the reader scale are visible.
b. Solution is turbid; lines on the reader scale are visible.
c. Solution is turbid; lines on the reader scale are not visible.
d. Solution is clear; lines on the reader scale are not visible.

65. (040) If the dithionite tube test is positive, what test is used to confirm results and provide additional information?

a. Sodium metabisulfite.
b. Hemoglobin (quantity in g/dl).
c. No further test is necessary.
b. Hemoglobin electrophoresis.

66. (041) Rouleaux formation and RBC aggregation are affected by the presence of plasma protein levels of the following examples except

a. fibrinogen.
b. alpha 1 globulin.
c. albumin.
d. alpha 2 globulin.

67. (041) If the ESR tube is not perpendicular during the test, the ESR

a. will not be affected.
b. will increase.
c. will decrease.
d. decreases rapidly, then increases.

68. (041) The effect of anemia on the erythrocyte sedimentation rate is described as

a. linear.
b. subject to correction.
c. inconsequential.
d. nonlinear.

69. (042) Performing the ESR more than 2 hours after the blood is drawn may produce which of the following effects?

a. Decreased ESR.
b. Increased ESR.
c. No effect.
d. 30 percent increase only.

70. (043) What would be the MCV if the hematocrit is 45 percent, the hemoglobin 15.0 g/dl, and the red cell count is 5,000,000 per mm^3?

a. 80 femtoliters (fl).
b. 85 femtoliters (fl).
c. 90 femtoliters (fl).
d. 95 femtoliters (fl).

71. (044) The degree of red cell fragility is directly related to what condition?

a. Pressure within the cell membrane.
b. Atmospheric temperature.
c. Atmospheric pressure.
d. Osmotic pressure.

72. (045) A general rule in the normal maturation of leukocytes is that, as the cells mature, the nuclear chromatin becomes more

a. clumped.
b. basophilic.
c. reticulated.
d. delicate.

73. (045) The appearance of only a few large, nonspecific azurophilic, cytoplasmic granules in a cell of the granulocytic series definitely identifies which of the following cells?

a. Myelocyte.
b. Myeloblast.
c. Promyelocyte.
d. Metamyelocyte.

74. (046) The differentiation of cells, more mature than the myelocyte, is based almost exclusively on what characteristic?

a. Cytoplasmic granules.
b. Nuclear configuration.
c. Nuclear chromatin.
d. Cell size.

75. (047) When granules dissolve in the process of staining and washing, and are water soluble, what type of leukocyte is suspected?

a. Eosinophils.
b. Basophil.
c. Segmented granulocyte.
d. Neutrophilic band.

76. (047) Which of the following cells may be identified by nuclear indentation more indented than the kidney shaped, but does not have filaments typical of segmented neutrophils?

a. Band granulocyte.
b. Neutrophilic metamyelocyte.
c. Basophilic metamyelocyte.
d. Eosinophilic segmented cell.

77. (048) The evaluation of megakaryocytes in bone marrow may be of importance in coagulation problems involving

a. plasmocytes.
b. thrombocytes.
c. normocytes.
d. myelocytes.

78. (048) The largest blood cell, ranging from 40-100 micrometers in diameter, is the

a. thrombocyte.
b. promegakaryocyte.
c. megakaryocyte.
d. megakaryoblast.

79. (049) The nuclear chromatin of the lymphoblast when compared with that of the lymphocyte and myeloblast is

a. finer, more delicate than the myeloblast.
b. coarser, more delicate than the myeloblast.
c. finer, not as delicate as the myeloblast.
d. coarser, not as delicate as the myeloblast.

80. (050) If overstained, the monocyte may be confused with the

a. metamyelocyte.
b. myeloblast.
c. megakaryoblast.
d. lymphoblast.

81. (050) When other differential features are absent, what may be used to classify a cell as a monocyte?

a. Diffused chromatin strands and the dull gray-blue cytoplasm.
b. Brainlike convolutions of the nucleus and gray-blue cytoplasm.
c. Diffused chromatin strands and the clear perinuclear zone in the cytoplasm.
d. Brainlike convolutions of the nucleus and clear perinuclear zone in the cytoplasm.

82. (051) Which of the following terms is used to describe crushed leukocytes resulting from mechanical pressure?

a. Phagocytic histiocytes.
b. Mast cells.
c. Stem cells.
d. Smudge cells.

83. (052) If the total leukocytic count is below 4,000 per mm^3, what term is used to describe this condition?

a. Leukopenia.
b. Leukocytosis.
c. Leukemia.
d. Neutropenia.

84. (052) A chronic infection which fails to stimulate the production of leukocytes is associated with which of the following conditions?

a. Neutropenia.
b. Eosinophilia.
c. Eosinopenia.
d. Neutrophilia.

85. (053) The classic Downey cells of infectious mononucleosis are reported as

a. hyperplasia.
b. polycythemia vera.
c. atypical lymphocytes.
d. leukopenia.

86. (053) How does the Downey classification of atypical lymphocytes correlate with the severity of mononucleosis?

a. There is 85 percent correlation.
b. There is 75 percent correlation.
c. The correlation is doubtful.
d. There is no correlation.

87. (054) In acute myelogenous leukemia, thin, eosinophilic, rod-shaped structures in the cytoplasm are called

a. toxic granulation.
b. Auer rods.
c. definitive granules.
d. Howell-Jolly bodies.

88. (054) What test is frequently used to differentiate a leukemoid reaction from myelogenous leukemia?

a. The leukocyte alkaline phosphatase test.
b. The leukocyte acid phosphatase test.
c. Peroxidase stain test.
d. Both peroxidase stain and acid phosphatase tests.

89. (055) A leukocyte containing ingested material, with a recognizable chromatin pattern and nuclear membrane, best describes

a. an L.E. cell.
b. a megalocyte.
c. a rosette.
d. a tart cell.

90. (055) When an engulfed pyknotic nucleus is observed in an L.E. preparation, what test condition is reported?

a. Positive; structures resembling L.E. cells.
b. Negative; structures resembling tart cells.
c. Positive for tart cells.
d. Negative for L.E. cells.

91. (056) What tissue does the intrinsic thromboplastin mechanism of coagulation involve?

a. Muscle.
b. Blood.
c. Subcutaneous.
d. Skin.

92. (057) Bleeding may be caused by all of the following conditions except

a. abnormalities among plasma proteins.
b. platelet disorders.
c. mechanical damage to the blood vessels.
d. polymerization of fibrin.

93. (058) The inherited deficiency of which factor is known as hemophilia A, or classical hemophilia?

a. X.
b. IX.
c. VIII.
d. VII.

94. (058) Which of the following factors, that is produced in the liver and does not require vitamin K for its production, is also called labile factor?

a. V.
b. VII.
c. X.
d. XI.

95. (058) Which of the following factors can be activated by contact with a glass surface?

a. I.
b. II.
c. XI.
d. XII.

96. (059) In stage 1, activation of Factor XII in vitro is accomplished in what manner?

a. Vascular injury.	c. Contact with glass.
b. Contact with collagen.	d. Contact with connective tissue.

97. (059) Prothrombin is converted to thrombin by combining

a. plasma thromboplastin with sodium ions.
b. plasma antithromboplastin with calcium ions.
c. plasma thromboplastin with calcium ions.
d. plasma thromboplastin with potassium ions.

98. (060) Tests commonly used to monitor heparin therapy are the

a. coagulation time and bleeding time.
b. coagulation time and prothrombin time.
c. activated partial thromboplastin time and the coagulation time.
d. activated partial thromboplastin time and the bleeding time.

99. (060) Which time test is used to monitor coumadin therapy?

a. Coagulation.	c. Bleeding.
b. Prothrombin.	d. Activated partial thromboplastin.

100. (061) Which of the following time tests encompasses all three stages of coagulation?

a. Activated partial thromboplastin.	c. Bleeding.
b. Coagulation.	d. Prothrombin.

101. (061) The thrombin time test measures what deficiency?

a. Fibrinogen.	c. The Hageman factor.
b. Function of proaccelerin.	d. Prothrombin consumption.

102. (062) What purpose does the fibrinolytic mechanism serve?

a. Keeps the vascular system free of fibrin clots.
b. Causes thrombosis.
c. Activates clot formation.
d. Keeps the vascular system free of plasmin.

103. (063) If a hemophilic man is married to a normal female, one would expect their

a. sons to be hemophilic.	c. sons to be carriers.
b. daughters to be hemophilic.	d. sons to be normal.

104. (063) Which of the following factors is used to confirm the diagnosis of hemophilia?

a. I.	c. VII.
b. II.	d. VIII.

105. (064) All of the following conditions are causes of acquired coagulation deficiencies except

a. bone marrow-hyperplasia.	c. hyperfunction of the spleen.
b. mass transfusions.	d. hemophilia B.

106. (065) The tourniquet test measures what deficiency?

a. Osmotic fragility.	c. A deficiency of fibrinogen.
b. Capillary fragility.	d. Intravascular deficiency.

107. (066) Bleeding time is dependent on all the following factors except

a. elasticity of the skin and capillary vessels.
b. efficiency of tissue fluids.
c. mechanical and chemical action of platelets.
d. presence of factor VII, stable factor.

108. (067) Which of the following factors may increase the clotting time?

a. Rough handling.
b. Blood quantities less than 1 ml.
c. Blood quantities greater than 1 ml.
d. Presence of tissue fluids.

109. (067) In thrombocytopenic purpura, the clotting time will show what results?

a. Increased.
b. Decreased.
c. Normal.
d. Slightly decreased.

110. (068) Clot retraction may appear increased if the patient shows a

a. decrease in plasma volume.
b. high fibrinogen level.
c. low fibrinogen level.
d. decrease in red cell mass.

111. (068) Incubation of a normal clot with the patient's plasma would reveal a defect in what constituent?

a. Fibrinolysin.
b. Prothrombin.
c. Fibrinogen.
d. Fibrin.

112. (069) In the Breaker-Cronkite method for platelet count, the blood is diluted with one percent

a. ammonium citrate.
b. potassium oxalate.
c. ammonium oxalate.
d. potassium chloride.

113. (069) In performing a platelet count, the most likely error occurs through

a. overshaking the pipette.
b. settling in the pipette.
c. settling in the chamber.
d. uneven distribution in the chamber.

114. (070) What three corrections are made in the electronic platelet count?

a. Coincidence, dilution, hematocrit.
b. Coincidence, dilution, RBC count.
c. Threshold, coincidence, hematocrit.
d. Threshold, coincidence, RBC count.

115. (070) In electronic counts, platelets entering the aperture at the same time as red cells will

a. be detected and cause a high count.
b. be detected and cause a low count.
c. not be detected and cause a high count.
d. not be detected and cause a falsely low count.

116. (071) How is the fibrinogen deficiency test affected by the presence of fibrinolysin?

a. The clot is partially or completely dissolved; test result is erroneous.
b. The clot is partially or completely dissolved; test result not affected.
c. Fibrinogen is converted to fibrin; test result is erroneous.
d. Fibrinogen is converted to fibrin; test result is not affected.

117. (071) In the semiquantitative method for fibrinogen assay, the fibrinogen is salted out with what reagent?

a. Ammonium chloride.
b. Ammonium oxalate.
c. Ammonium sulfate.
d. Sodium chloride and merthiolate.

118. (072) Why is the use of sodium oxalate not recommended for the prothrombin time?

a. Factor IV is labile in oxalated blood.
b. Factor V is labile in oxalated blood.
c. Factor VII is labile in oxalated blood.
d. Factor VIII is labile in oxalated blood.

119. (072) Control values obtained on each set of prothrombin time determinations should fall within

a. ±0.5 seconds of each run.
b. ±10 seconds of each run.
c. ±15 seconds of each run.
d. ±20 seconds of each run.

120. (073) After removal of the serum from the clot, how long should the serum be held?

a. May be stored indefinitely at 4° C.
b. May be stored indefinitely, frozen.
c. Must not be held for more than 2 hours at 4° C.
d. Must not be held for more than 6 hours at 4° C.

121. (073) The Simplastin A® used in the prothrombin consumption test supplies all of the following except

a. tissue thromboplastin.
b. calcium.
c. factor VIII (stable factor).
d. fibrinogen.

122. (074) In the regular partial thromboplastin time, activation is controlled by all of the following except

a. glassware surface.
b. tissue in contact with the glass.
c. type of syringe.
d. kaolin.

123. (075) The thromboplastin generation test measures the efficiency with which

a. extrinsic thromboplastin is formed.
b. intrinsic thromboplastin is formed.
c. extrinsic thromboplastin is converted.
d. intrinsic thromboplastin is converted.

124. (075) Which of the following reagents in the thromboplastin generation test serves as a platelet-like substance?

a. Barium sulfate.
b. Calcium chloride.
c. Thromboplastin.
d. Normal plasma substrate.

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CDC 90413

MEDICAL LABORATORY TECHNICIAN—HEMATOLOGY, SEROLOGY, BLOOD BANKING AND IMMUNOHEMATOLOGY

(AFSC 90470)

Volume 2

Laboratory Procedures in Blood Banking and Immunohematology

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THIS PUBLICATION HAS BEEN REVIEWED AND APPROVED BY COMPETENT PERSONNEL OF THE PREPARING COMMAND IN ACCORDANCE WITH CURRENT DIRECTIVES ON DOCTRINE, POLICY, ESSENTIALITY, PROPRIETY, AND QUALITY.

Preface

THIS SECOND volume of CDC 90413 is concerned with blood banking and immunohematology. Chapter 1 discusses antigen and antibody reactions in their relationship to blood banking. Reference is made to the molecular structure and basic characteristics of antibodies frequently encountered in blood banking. Major blood group systems are discussed in Chapter 2. The special precautions and procedures used in preparing blood components for transfusion is presented in Chapter 3. A section of this chapter is devoted to suggestions for maintaining quality assurance in blood banking. The last chapter of this volume is devoted to the operation of a blood donor center and briefly describes policies and guidelines of the Air Force Blood Program.

Some of the material has been illustrated in foldouts 1 and 2. When the text discusses this material, open the foldout at the back of the text and refer to it as you study.

A glossary of technical terms is included in Volume 3.

Please note that in this volume we are using the singular pronoun *he*, *his*, and *him* in its generic sense, not its masculine sense. The word to which it refers is *person*.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to School of Health Care Sciences/MSTW, Sheppard AFB TX 76311. NOTE: Do not use the suggestion program to submit corrections for typographical or other errors.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Behavioral Objective Exercises, Volume Review Exercise, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFS AL 36118, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 36 hours (12 points).

Material in this volume is technically accurate, adequate, and current as of December 1976.

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CHAPTER 1

NOTE: In this volume, the subject matter is developed by a series of Learning Objectives. Each of these carries a 3-digit number and is in boldface type. Each sets a learning goal for you. The text that follows the objective gives you the information you need to reach that goal. The exercises following the information give you a check on your achievement. When you complete them, see if your answers match those in the back of this volume. If your response to an exercise is incorrect, review the objective and its text.

Immunohematology

BLOOD HAS FASCINATED men since the first caveman noted that loss of blood meant the end of life. Early attempts to restore blood to the body by replacing it with blood from animals or other human beings most often resulted in death. Medical practice of centuries past often called for bleeding the patient as a means of therapy. It was not until the end of the 19th century that certain mysteries of life were explained through the sciences of genetics and immunology, which emerged to form the basis for immunohematology.

Immunohematology is the study of the immune response, with particular reference to blood. Through the science of immunohematology we have learned to administer blood with the reasonable assurance that it will support life rather than end it. We have also learned much about blood disorders, particularly hemolytic disease due to antigenantibody interaction.

1-1. Genetics and Immunology

The foundation for the study of immunohematology is genetics, the study of heredity and inheritance. Human red blood cells are produced under the control of genes. All physical and chemical structures of the body, including characteristics of the red blood cells, are determined by gene inheritance. Blood banking is a practical application of genetic principles. As you read this chapter and the remainder of the volume, certain terms may be unfamiliar to you. For this reason, a glossary is provided at the end of Volume 3.

200. Identify genetic terms and basic principles of genetics as applied to blood groups, sex-linkage, and other inherited characteristics.

Genetics. When we type blood with antisera, we are identifying a phenotype. A phenotype is the physical expression of a trait. Blue eyes, blonde hair, and group A blood are all examples of phenotypes. A genotype is an expression of the nature, number, and arrangement of genes, which are, of course, the biochemical entities that determine the phenotype. For example, group A blood, which is a phenotype, may be expressed by the genotype AO or AA. In the clinical laboratory there is no practical way to observe the genes themselves, and we must therefore be content with observing the related effect of gene action. Antiserum reacts with a protein (antigen) on the red blood cell. The presence of the antigen is under direct genetic control, but we do not type for the gene with antiserum. We are testing for evidence of the presence of one or more genes.

Various genes control the nature of antigens, which occur on a person's red blood cells. The offspring develops red blood cell antigens according to genes inherited from his parents. Let us briefly review the theory of gene inheritance as it applies to the subject at hand.

Inheritance theory. **All human cells contain a complement of chromosomes consisting of 22 pairs of autosome and 1 pair of sex chromosomes. The sex chromosomes comprise an uneven pair, with a large chromosome being referred to as X and a small chromosome as Y. If two X chromosomes are inherited, the sex of the offspring is female. If an X and Y are inherited, the sex is male.**

Sex-linked characteristics. With the exception of factor Xg, blood group genes are not sex-linked. This gene is indicated by its presence on the X chromosome, as an antigen called Xg^a. The Xg factor is said to be sex-linked. In this case, only the females have two genes for the trait. Other genes of interest to the blood bank technician also show sex linkage, including the gene for hemophilia and color blindness, which are carried on the X chromosome. A male manifests hemophilia if he has one gene for the condition. Two genes are required to produce the disease in the female. Such a genetic condition is usually fatal to the ovum. Except for certain sex-linked traits, a person has two or more genes for each antigen characteristic of his blood cells.

Remember that the female receives two doses of each sex-linked genes (one from each parent), while the male receives only one dose, which is always from the mother. It can thus be said that in the male all sex-linked genetic characteristics are inhertied from the mother.

Linkage is not limited to the sex chromosomes. Two genes close to each other at a locus before meiosis (reduction division) tend to be inherited together. This is of practical value because the presence of a trait, known to be linked to another trait, is evidence that a gene is present for the other linked trait as well. This explains why a correlation exists between blood groups and certain hereditary abnormalities. For example, the chance that a person of blood group O will develop a duodenal ulcer is nearly 1.5 times as great as the possibility of the same disorder in someone of the other blood groups. This is because the secretion of gastric juice is controlled by a gene in close proximity to the O gene. A similar comparison can be made with some other disease processes.

Exercises (200):

Match each of the following terms in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

_____ 1. A physical expression of a trait. For example, individuals whose blood we type as group A.
_____ 2. The actual gene combination that is inherited. A group A individual may have a gene combination of AO or AA.
_____ 3. The larger of the sex chromosome pair.
_____ 4. The smaller of the sex chromosome pair.
_____ 5. The sex of the offspring if two X chromosomes are inherited.
_____ 6. The sex of the offspring if an X and a Y are inherited.
_____ 7. All human cells contain a complement of chromosomes consisting of 22 pairs of these.
_____ 8. A term referred to genes which carry factor Xg group hemophilia and color blindness.
_____ 9. Number of genes required to produce hemophilia in males.
_____ 10. Number of genes required to produce hemophilia in females.
_____ 11. Receives only one dose of sex-linked genes which is always from the mother.
_____ 12. Secretion of gastric juices is believed to be controlled by a gene in close proximity to this gene.
_____ 13. In the male, all sex-linked genetic characteristics are inherited from this parent.

Column B

a. Mother.
b. Female offspring.
c. Five.
d. Y chromosomes.
e. A sex-linked.
f. Phenotype.
g. Male offspring.
h. Two.
i. Father.
j. X chromosome.
k. One.
l. Ĝenotype.
m. O gene.
n. A gene.
o. Autosomes.

201. Identify related genetic terms of the ABO and Rh systems and the possible results of given genotype combinations.

Pattern of Genetic Inheritance—ABO System. The antigens of the ABO system are inherited according to Mendelian laws. The A and the B genes are codominant or equally dominant and may be considere as being dominant to the O gene which is an amorph. An amorph does not produce a detectable antigen. If a person is of blood group A, he has either one or two genes for this trait. The group A individual may be AA or AO (genotype) and still be of blood group A (phenotype). If both genes are alike, for example, AA, the person is homozygous. If the genes are not alike, for example, AO, the individual is heterozygous. When this person produces offspring, he passes on only one of the two genes. The same is true of the other parent. The offspring becomes the recipient of two genes, and his blood group depends upon the genes he inherits. Figure 1-1 illustrates the possibilities with regard to the ABO systems for parents who are both of the same blood group. Of course, parents may be of different blood groups, but the rules of inheritance are the same. Following the reasoning in figure 1-1, can you predict the possible blood group(s) of offspring from parents in different blood groups? Try writing out some of the possibilities.

Each gene of a particular pair or group is an allele to the other(s). For instance, in the genetype AO, A and O are alleles to each other. Alleles occur at corresponding positions or loci on homologous (matching) chromosomes. In basic genetics, the concept of dominance and recessiveness is well known. This means that one gene may be dominant over an allele in the effect that is produced. For example, if the gene fo dark eyes (B) is dominant over the gene for blue eyes (b), a person who possesses both genes (Bb) will have dark eyes rather than blue eyes. For all practical purposes, genes that control blood groups are neither dominant nor recessive to each other. While it is true that a person who possesses an O gene in addition to either A or B will be grouped as A or B on the basis of cell antigens, the influence of the O gene is there. It simply cannot be detected with available typing sera. A single locus on a chromosome contains an A, B, or O gene, but no more than one of them. You may use the Punnett Square method illustrated in figure 1-2 to assist you in predicting the possibilities of an offspring.

Pattern of Genetic Inheritance—Rh System. The Rh system follows the same pattern of genetic inheritance. There are six genes in the Rh system (C, c, D, d, E, e,). The Rh genes from each parent remain together on the same chromosome during fertilization. Thus, the Rh genes on a given chromosome in the offspring represent the same arrangement as those inherited from one of the parents. For example, if the Rh gene grouping on a chromosome of the mother is CDe and the group of Rh genes on a chromosome in the sperm is cde, the offspring is CDe/cde, not some other combination thereof. What is perhaps more complicating in the Rh system is that several genes are involved, rather than the single *Rh factor*, as originally proposed. If you consider the D factor, the genes are D(Rh_o) and d(Hr_0). Unfortunately, we do not have anti-d typing

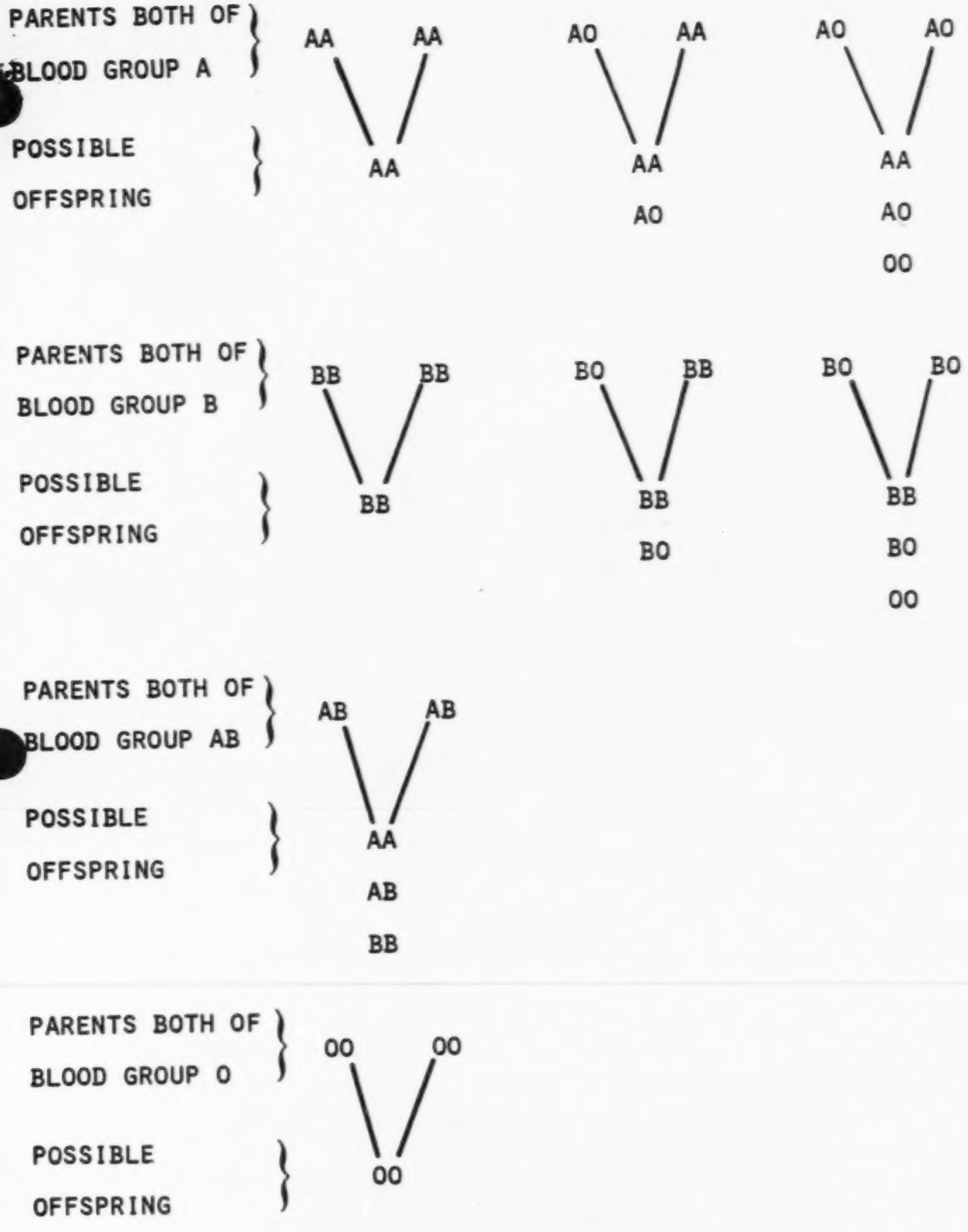
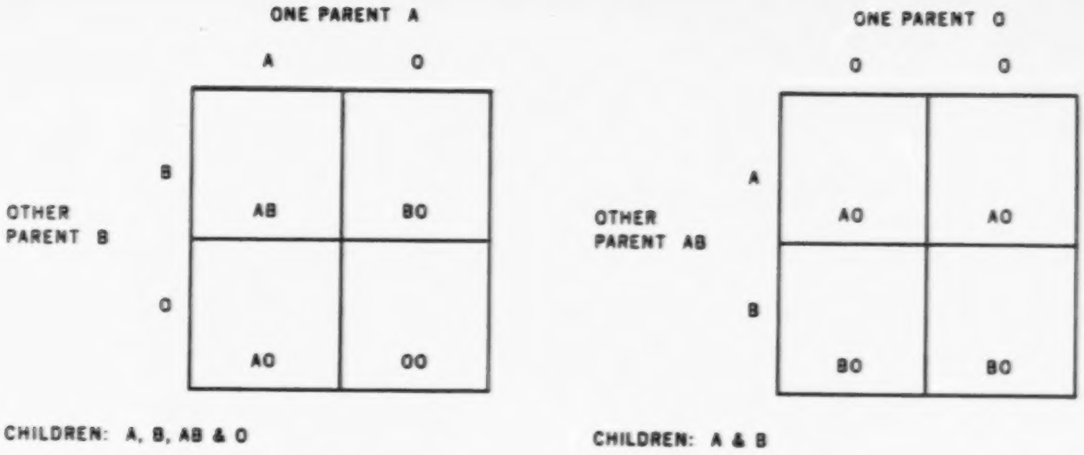

PARENTS BOTH OF BLOOD GROUP A
POSSIBLE OFFSPRING
AA AA
AA
AO AA
AA AO
AO AO
AA AO OO
PARENTS BOTH OF BLOOD GROUP B
POSSIBLE OFFSPRING
BB BB
BB
BO BB
BB BO
BO BO
BB BO OO
PARENTS BOTH OF BLOOD GROUP AB
POSSIBLE OFFSPRING
AB AB
AA AB BB
PARENTS BOTH OF BLOOD GROUP O
POSSIBLE OFFSPRING
OO OO
OO

Figure 1-1. Inheritance of ABO blood groups.



ONE PARENT A

OTHER PARENT B	A	O
B	AB	BO
O	AO	OO

CHILDREN: A, B, AB & O

ONE PARENT O

OTHER PARENT AB	O	O
A	AO	AO
B	BO	BO

CHILDREN: A & B

Figure 1-2. Punnet squares—ABO blood group inheritance.

serum, and there is consequently some question of gene action in this case. Again, you may use Punnett Squares to determine the possible gene combination of an offspring by using figure 1-3. We will discuss the Rh system further in Chapter 2 of this volume. At this point, you should concentrate on the theory of inheritance and its application to blood groups.

Determination of genetic patterns—for example, genotype—is of value in prenatal studies, medicolegal cases, and in locating blood for sensitized patients. In prenatal studies, the possibility of maternal isoimmunization to Rh antigens of the infant requires detailed phenotyping of the mother. The phenotype is then used to derive the probable genotype. It is also desirable to type the father of the child in order to determine which antigens may be inherited by the infant. At the present, there is a problem in predicting inheritance based on typing the parents. For example, we have no certain way of knowing whether a parent is homozygous for D, (for example, DD) or whether he is heretozygous (Dd), unless we have access to genealogical studies of the same family. However, we can type for C, c, E, and e, as well as D, in terms of their red blood cell antigens. Once we have determined which genes are present, it is a matter of probability whether the parent is homozygous or heterozygous. Table 1-1 gives the probability of common Rh positive genotypes. The subject of Rh isoimmunization is further explained in Chapter 2.

Exercises (201):

Match each item in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item it most nearly describes. Each element in column B may be used once or more than once.

Column A

_____ 1. A gene which does not produce a detectable antigen.

_____ 2. Pairs of genes; one inherited on a chromosome in the ovum, the other on an identical chromosome in the spermatazoan.

_____ 3. Genes that are not alike.

_____ 4. Genes that are alike.

_____ 5. Possible genotype combinations if one parent is AO and the other is AB.

_____ 6. Possible geneotype combination if one parent is AA and the other is AO.

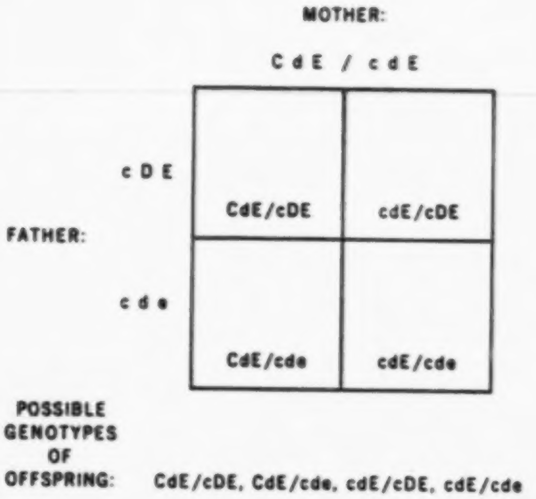
_____ 7. Positions on homalogous chromosomes.

_____ 8. Possible genotype combinations if a mother's Rh gene type is CDE/cDe and the father's genotype is cDE/cde.

_____ 9. Equally dominant.

Column B

a. Alleles.
b. Homozygous.
c. Amorph.
d. Heterozygous.
e. Loci.
f. AA, AO.
g. AA, AO, AB, BO.
h. CdE/cDE, CdE/cde, cdE/cDE, cdE/cde.
i. CDE/cDE, CDE/cde, cDe/cDE, cDe/cde.
j. Codominant



MOTHER: CdE / cdE

FATHER:	CdE	cdE
cDE	CdE/cDE	cdE/cDE
cde	CdE/cde	cdE/cde

POSSIBLE GENOTYPES OF OFFSPRING: CdE/cDE, CdE/cde, cdE/cDE, cdE/cde

Figure 1-3. Suggestion for determining possible genotypes from given gene combinations.

TABLE 1-1
PROBABLY OCCURENCE OF THE *COMMON D GENOTYPES*

Antigen Present						Approximate Percent of Population
D	C	E	c	e		
+	+	-	+	+	DCe/dce	33.0
					DCe/Dce	2.0
+	+	-	-	+	DCe/DCe	18.0
					DCe/dCe	0.8
+	+	+	+	+	DCe/DcE	12.0
					DCe/dcE	1.5
					DcE/dCe	1.5
+	-	+	+	+	DcE/dce	11.0
					DcE/Dce	1.0
+	-	+	+	-	DcE/DcE	2.0
					DcE/dcE	0.3
+	-	-	+	+	Dce/dce	2.0
					Dce/Dce	0.1

202. Cite ways in which principles of genotyping and phenotyping may be used.

Applications of Genotyping and Phenotyping Principles. In medicolegal cases, it is often possible to rule out paternity by applying the hereditary (Mendelian) law. It can sometimes be stated on the basis of genotype that a particular individual could *not* be a parent of a child. Can you imagine how a group AB father and a group A mother could have a group O child. Of course, they could not. On the other hand, it is never possible to prove that someone *is* the father of a particular child based on genetic studies. Other uses of genotyping in legal matters include the identification of blood samples associated with crime.

Determining genotype is sometimes essential in the location of blood for sensitized patients. For example, a female patient who has been sensitized against c (possesses antibodies against c antigen) should receive blood which is negative for the c antigen. In this case, we phenotype using anti-c serum, and from this we derive the possible genotype. Further, one does not restrict the search for type-specific blood to patients who have been sensitized. Patients should never receive blood cell antigens that could sensitize (immunize) them. Keep in mind that c is usually most antigenic, next to D and D^u in the Rh series. Also keep in mind that *it is never wise to transfuse a female with blood from her husband.* She may be sensitized to antigens that are also possessed by the fetus if she should become pregnant.

Exercises (202):

1. How could a group AB father and a group A mother be the parents of a group O child?

2. How is it possible to prove that someone is the father of a particular child based on genetic studies?

3. In locating blood for a sensitized patient, which process is essential?

4. A female patient who has been sensitized against the c antigen should receive blood which is essentially negative for what antigen?

5. How do we derive the possible genotype in case of c antigen?

6. Next to D and D^u in the Rh series, which antigen is considered most antigenic?

7. Why is it never wise to transfuse a female with blood from her husband?

203. Cite the basis for immunology and blood group serology and identify the properties and characteristics of antigens and related terms.

Immunology. Immunology, usually defined as the study of resistance to infectious disease, has broadened in scope through its subdivision serology. The *basic concepts* of antibodies elicited by the infectious disease process laid a foundation for present thinking in immunology, and have been developed through the study of blood groups and their serologic reaction. A knowledge of the basic principles of immunology is necessary for a true understanding of blood transfusion serology. For example, of prime consideration are the blood group factors which are antigens, and their reactions with corresponding antibodies which constitute antigen-antibody reactions. Reactions between the very specific substances known as antigens and their corresponding antibodies are the basis of immunology and blood group serology. Let's review some basic and essential properties and characteristics of antigens.

Properties of Antigens. For the purposes of blood banking, antigens and antibodies are best described at the molecular level. Most antigens are protein in nature, although on occasion, some may be carbohydrates, such as pneumococcal polysaccharides. Carbohydrates appear to show some measure of antigenicity. Rarely do antigens appear as lipids. Antigens are often a combination of a carbohydrate portion referred to as "*hapten*" or antigenic determinant group, coupled with a protein carrier. The hapten does not produce antibodies of itself, but once antibody is formed the hapten is capable of combination with the antibody produced and will determine specificity. Antibodies are usually produced in an animal that lacks the antigen. When antigenic substance from one species is injected into another species and antibodies are produced, the process is known as *heteroimmunization*. When the antigen produces antibodies in the same species it is called *isoimmunization*. Sites on the antigen comprise a region called the antigenic determinant. Several molecules will link together at these complementary sites in a lattice arrangement during antigen-antibody reactions. Antigenic determinants on a red blood cell with complementary regions on the antibody combining sites are hypothetically illustrated in figure 1-4. The complementary regions on the antibody are the antibody combining sites. There are at least two, and often many more, of these combining sites per antibody mulecule.

Antigen Characteristics. Antigens that are found in some members of a species but not in other members are called isoantigens. Likewise, immunization that occurs in response to an isoantigen is referred to as isoimmunization. Immunization due to blood transfusion is an example of isoimmunization. If an antigen is introduced into the body and the person does not possess the corresponding antibodies, there is usually no immediate reaction. About 2 weeks later, however, antibodies can be detected in the circulation if antigenic stimulation has taken place. (Some antigens apparently do not cause a measurable response.) If the person subsequently receives another dose of the same antigen, the immune response is more rapid, and the antibody titer will rise higher and remain at that

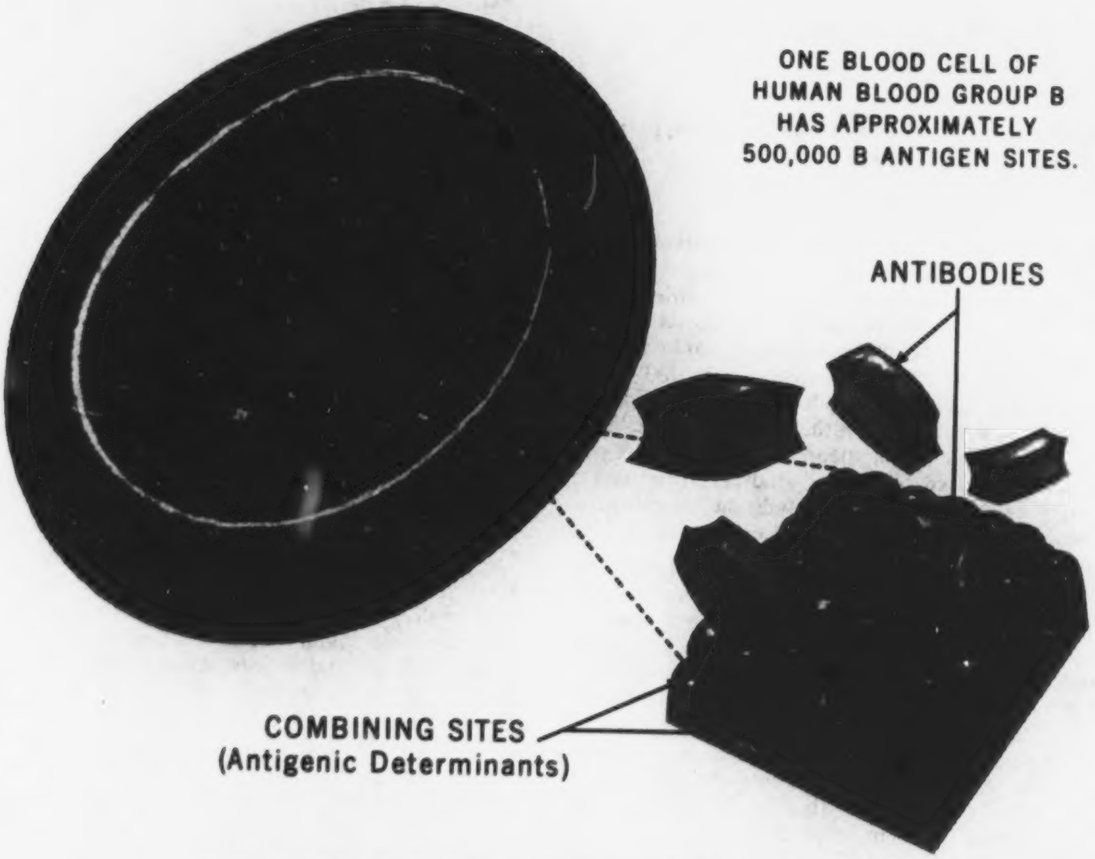


Figure 1-4. Antigenic determinants on a red blood cell (antibody combining sites).

level for a longer period. Antibodies due to a secondary response may also have greater combining properties with their specific antigens. It is interesting to note that the secondary response can be caused by antigens that are related but not necessarily identical to those that evoked the primary response. Isoimmunization is the reason it is often difficult to find compatible blood for a person who has received multiple transfusions over a period of time. Other causes of sensitization include complications of pregnancy (for example, transfer of antigens from fetal to maternal circulation) and other antigenic stimuli, including the injection of horse serum and other animal protein preparations. As far as is known, Rh antigens, specifically, may be introduced into a person only through transfusion or pregnancy.

Exercises (203):

1. What is the basis of immunology and blood group serology?

2. What constitutents comprises the nature antigens?

3. Antigens are often combination of what two parts?

4. What part of the antigen determines the specificity?

5. When antigenic substances from one species is injected into another resulting in antibody production, the process is called ______________ .

6. Sites on the antigen comprise a region called the ______________ .

7. Antigens that are found in some members of a species but not in other members are called ______________ .

8. Immunization due to blood transfusion is an example of ______________ .

Questions 9 through 12. Indicate whether each of the following sentences if true (T) or false (F). If you indicate false, explain your answer.

T F 9. If a person who has antibodies received another dose of the same antigen, the immune response is less.

T F 10. The antibody titer of a person who has received another dose of the same antigen will remain normal.

T F 11. Antibodies due to a secondary response may have greater combining properties with their specific antigens.

T F 12. It is often difficult at times to find compatible blood for a person who has received multiple transfusions over a period of time when isoimmunization has occurred.

204. Define antibodies and immunoglobulins and state the characteristics and properties of both.

Characteristics of Antibodies. Antibodies are specific serum proteins produced in the lymphoid tissue as a result of stimulation with an antigen. Antibodies are capable of chemical union both *in vivo* and *in vitro* with the particular antigen responsible for their production. They are usually found in plasma, although some kinds of antibodies do not appear to circulate but are fixed to body tissues or cells. Almost all antibodies are in the gamma globulin fraction of the serum proteins, but a few are nongamma proteins. Electrophoretic separation of proteins shows antibody activity to be associated with those fractions found in the gamma globulin regions. Other methods, such as ultracentrifugal analysis, diffusion, immuno-electrophoresis, which involves not only electrophoretic mobility but also the size, shape, and antigenic structure of the molecules, have demonstrated that there are with certainty many different forms of antibody globulin.

Immunoglobulins. The term "immunoglobulin" applies collectively to all protein molecules having antibody activity, for example, the ability to combine or react specifically with antigen, or structurally related protein molecules such as Bence-Jones proteins, myeloma proteins, and Walderstrom macroglobulins that are found in various pathological conditions.

Classes of Immunoglobins. There are at present five distinct immunoglobulin classes distinguishable on the basis of chemical, physical, and biological characteristics and are referred to as Ig. These are called IgM, IgG, IgA, IgD, and IgE. Most circulating antibodies are IgG or IgM, and together these make up all of the classic gamma globulin fraction of plasma protein.

Immunoglobulin A (IgA) is the antibody found in body secretions such as tears and nasal secretions. IgD and IgE have been identified only recently. They are present in trace amounts, and little is known about their biologic importance. Look at table 1-2 and note some of the characteristics of the immunoglobulins. It should be noted that in terms of their sedimentation rate during centrifugation at high speed, antibodies are often labeled in Svedberg (S) units. There is, of course, a relationship between molecular weight and the sedimentation constant. Blood group antibodies of the Rh, K, and Jk systems are usually 7-S globulins. A newer term for 7-Sγ globulins is γG or IgG. In general, *naturally occurring* antibodies of the Le, MNS, P, and certain other systems are macroglobulins of the 19-S variety. Synonyms for 19-S globulins are γM and IgM. These are the two principal groups of immunoglobulins (IgG and IgM) of importance to the immunohemotologist. A third group, γA or IgA, are also 7-S immunoglobulins. These are implicated in the Rh system.

Exercises (204):

1. Define the term "antibodies."

2. In what fraction of serum proteins are antibodies found?

3. What methods of detection and analysis have indicated the existence of many different forms of antibody globulin?

4. To what type of proteins does the term "immunoglobulin" apply?

5. What are the five immunoglobulins?

6. Immunoglobulins are distinguishable on the basis of what three characteristics?

7. Which immunoglobulin is found in body secretions, such as tears or nasal secretion?

8. There is a relationship between the molecular weight and what other characteristic?

9. What type of immunoglobulin are blood group antibodies of Rh, K, and Jk systems?

10. Antibodies of the Le, MNS, P, and certain other systems are macroglobulins associated with which immunoglobulin?

205. Point out the components of the basic immunoglobulin structure and state their significance.

Structure of Immunoglobulin. Immunoglobulin molecules are composed of one or more basic structural units that are common to all classes of immunoglobulins. The basic structural units are composed of four polypeptide chains: two identical H or heavy chains with a molecular weight of 50,000 to 70,000, and two identical light chains with a molecular weight of 20,000 to 25,000. Note figure 1-5. The four chains are joined by three disulfide bonds shown. The light chains are of two types common to all classic and are designated as kappa (κ) or lambda (λ), but only one of these is associated with any individual antibody. Differences in the heavy chains account for most differences found among the various types of immunoglobulins. In addition, each of the four chains has a terminal region in which the amino acid sequence varies. Figure 1-5 shows that this region is called the *variable* region and forms the antigen binding site. The sequence of amino acids in the variable region determines the exact specificity of the antibody.

Exercises (205):

1. In the basic arrangement of each immunoglobulin, what are the large polypeptide chains called?

2. The larger chains are joined to two smaller polypeptide chains called ____________________.

3. What is the molecular weight of the smaller polypeptide chains?

4. What are the two types of light chains common to all units called?

TABLE 1-2
SOME PROPERTIES AND CHARACTERISTICS OF THE IMMUNOGLOBINS

Present Name	IgG	IgM	IgA	IgD	IgE
Molecular Weight	145,000	900,000	160,000	150,000	150,000
Sedimentation constant (Svedberg units)	7S	19S	7S, 13S, 15S, 17-18S	7S	8S
Synonyms	Gamma G, γG, 7S, 7Sγ, γ_2, γss, 6.6S, 7Sγ_2	Gamma M, γM β_2M, 18S, 19S, β_2-C, iota-globulin, macroglobulin	Gamma A, γA, β_2A, γ_1A, 7S-13S, 7S-14Sγ_1, β_x	Gamma D, γD, γ_2D	Gamma E, γE
Carbohydrate content	2.5%	10.0%	10.0%	unknown ?	10.7%
Normal serum level (mg/dl)	700-1,500	150-250	60-170	.003-0.03 (mg/ml)	.0001-.0006 (mg/ml)
Half-life (days)	30	5.1	5.8	2.8	2.3
Electrophoretic mobility	γ_2 to α_2; γ	fast γ; slow β_2	γ_1 to α_2 fast; slow β	γ to β; γ and β	γ_2 to α_2; γ and β
Cross Placenta	Yes	No	No	?	?
Antibodies	Major antibacterial, antiviral, and antitoxic; sensitizing to Rh; warm, incomplete, blocking; IgG$_1$ and IgG$_3$ fix complement well, IgG$_2$ weakly, IgG$_4$ not at all, antinuclear	Natural heterophil; ABO isoantibodies; rheumatoid factor; antinuclear; cold agglutinins; certain Rh complete saline	Bacterial agglutinins, skin sensitizing, cold agglutinins, isoagglutinins, anti-insulin, antinuclear (?)	?	Neutralizes allergens; fixes to skin; human skin sensitizing; myeloma protein

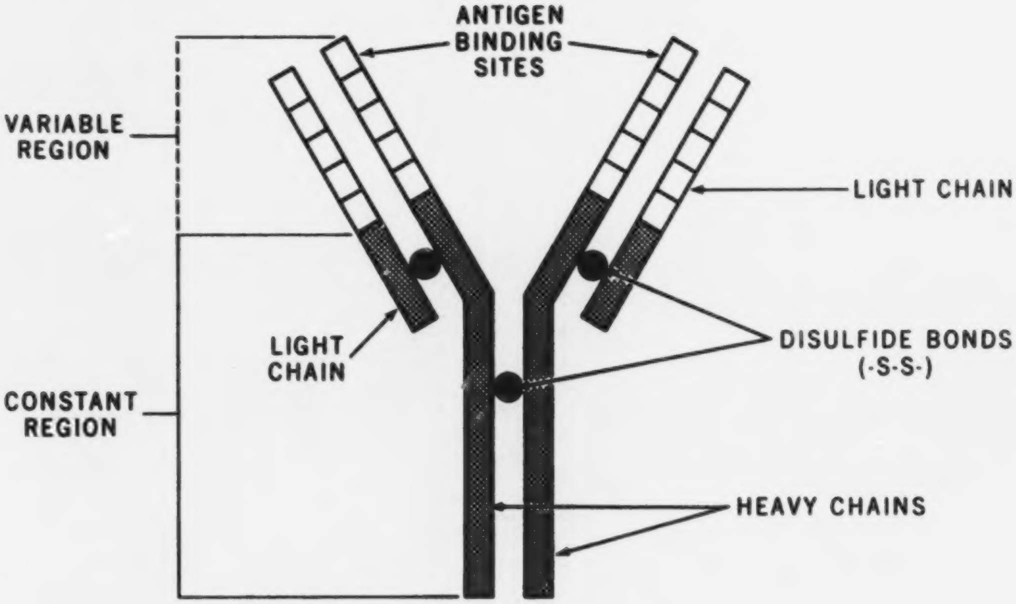


Figure 1-5. Basic immunoglobin structure—IgG molecule.

5. What accounts for most differences found among various types of immunoglobulins?

6. By what substance are the four chains joined together?

7. What region on the unit forms the antigen binding site?

8. What determines the exact specificity of the antibody?

206. Indicate whether the given statements correctly reflect the conditions responsible for a primary response and secondary or anamnestic response in antibody formation.

Primary Response. After a suitable antigen is injected or enters the body, a complex series of reactions leads ultimately to the production of an antibody. If the animal has never been exposed to the antigen or related one, the response is termed "primary." Even though there are macrophages located through the body, those in the lymph nodes and spleen appear particularly essential to the antibody synthesizing mechanism. It is believed that the vital role of these cells is probably that of *processing* the antigen, for example, absorbing it and converting it into a form such as ribonucleic acid (RNA) which can be passed on to the lymphoid cells where antibody is actually made. Plasma cells are the most active producers of antibody. The sequence of events requires time during which there is a lag of about 7 to 10 days, known as the *induction period.* Even though IgM (19-S) and IgG (7-S) antibodies are probably formed, only the IgM can be demonstrated during the early stages of antibody production. Nevertheless, continued production is of the IgG (7-S) type. The peak of antibody production will be reached in 10 to 30 days dependent on such factors as the animal immunized, the antigen itself, the amount of antigen used, and many others. In the case of blood group antigens, antibody production continues for lengthy periods of time, whereas significant amounts of antibody are present for years.

Secondary or Anamnestic Response. The first injection of antigen causes the antibody-producing cells to develop a *memory.* Thus, the second time when the same injection or related antigen takes place, a response follows that is different from the primary one. The lag time is decreased from 10 to 1 day or even less. Further, more antibody is produced, causing high titers to be reached rapidly. The antibody produced in such manner has better combining quality.

Exercises (206):

Indicate whether each of the following sentences is true (T) or false (F). If you indicate *false*, explain your answer.

T F 1. If an animal has never been exposed to an antigen or related one, the response is termed "anamnestic."

T F 2. Microphages in the lymph nodes and spleen are particularly essential to the antibody synthesizing mechanism.

T F 3. The antigen is absorbed and converted into a form such as DNA.

T F 4. Plasma cells are the most active producers of antibody.

T F 5. The sequence of events during which time there is a lag of 7 to 10 days of antibody formation is called the *reduction* period.

T F 6. During the early stages of antibody formation, only the IgM antibody can be demonstrated.

T F 7. The first injection of antigen causes the antibody-producing cells to develop an anamnestic response.

T F 8. In the anamnestic response, the lag time is increased from 1 to 10 days.

T F 9. The antibody produced as a result of a secondary response causes high titers to be reached rapidly.

T F 10. Compared to that of a primary response, the antibody produced as a result of a secondary response has poor combining capacity.

207. Identify the given antigen-antibody reactions in terms of the types, causes, and sources of false reactions.

In Vivo and In Vitro Reactions. Antigen-antibody reactions can take place *in vivo* or *in vitro*. In vivo reactions usually interfere with the invasion of the foreign antigens and in so doing protect against disease. In autoimmune states *in vivo* antigen-antibody reactions may actually cause disease such as acquired hemolytic anemia or idiopathic thrombocytopenic purpura. The antigen-antibody reactions that the blood bank technician observes are *in vitro* reactions. These reactions are of great interest and importance to the technician because it is through them that antigens or antibodies can be detected and studied. Agglutination and hemolysis are the two general types of *in vitro* reactions that the technician observes in the laboratory. First let's consider agglutination.

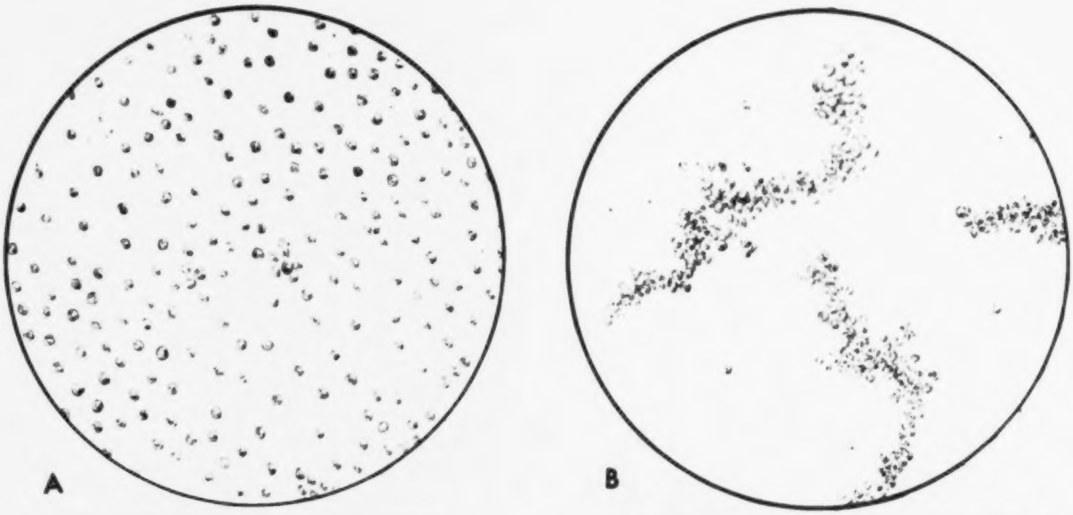
Agglutination. Agglutination, or the clumping together of cells, is illustrated in figure 1-6,B and C. In reading agglutination, it is necessary to rule out rouleaux.

Pseudoagglutination. As you already know, pseudoagglutination or rouleaux formation is a type of false agglutination in which the red cells stack together, as shown in figure 1-6,D. When rouleaux is observed macroscopically, the cells tend to tumble and slide down when the glass slide or tube is tilted. True agglutinations are more granular and less regular than rouleaux. To eliminate rouleaux, add saline to the reaction mixture, one drop at a time, until the adhering cells disperse. False agglutination results from abnormal proteins or factors resulting from medication. We will mention the phenomenon of rouleaux again in treating sources of error associated with compatibility testing.

Panagglutination. Panagglutination is bacteriogenic agglutination resulting from bacterial action and does not occur as a rule when fresh, sterile sera are used. Bacteriogenic agglutination can occur in certain patients with sepsis whose red cells have become polyagglutinable.

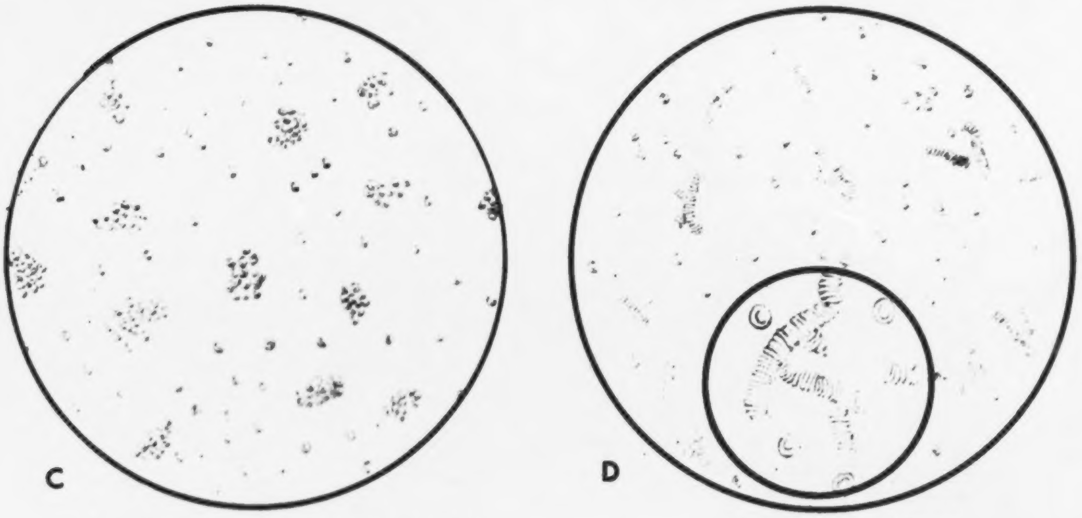
Polyagglutination. Polyagglutination is associated with infections by micro-organisms and may occur *in vivo* or *in vitro*. The *in vivo* infection may so alter the cells causing them to be agglutinable by many sera.

Autoagglutination. Autoagglutination is the nonspecific clumping of an individual's cells by factors in his own plasma or serum. Autoagglutination is most common at low temperatures (for example, at 5° C), and therefore, such antibodies are called *cold agglutinins.* You will see this phenomenon in primary atypical pneumonia and acquired hemolytic anemia, among other conditions. As a rule, cold agglutination is a reversible reaction. That is, when the test materials are warmed, the agglutination rapidly disperses. However, agglutination due to specific hemogglutinins does not disappear after warming.



FREE UNAGGLUTINATED BLOOD CELLS

GROSS AGGLUTINATION OF BLOOD CELLS



WEAK AGGLUTINATION OF BLOOD CELLS

ROULEAUX FORMATION

Figure 1-6. True and false agglutination.

Other Sources of False Agglutination. Red cells agglutinate as the result of a two-phase process. First, antibodies combine with antigen molecules at specific sites on the cell surface. Second, the antibody-coated cells clump as they come into contact with each other. Agglutination proceeds faster if the contact of the cells is increased through centrifugation, stirring, or agitation. Thus, in laboratory test procedures, we centrifuge at specific times to force the cells together. False agglutination is a danger if the centrifugal force packs the cells too tightly, causing the suspension to give the appearance of clumping without an antigen-antibody reaction.

If fibrin or other enmeshing substances are present in a mixture of cells and serum, the red cells can become trapped and the particles have the appearance of agglutination. It is therefore important to carefully control the time and speed of centrifugation. The speed and time should be such that normal cells disperse readily; yet centrifugation should be adequate to allow complete clumping when erythrocytes are mixed with very small amounts of specific antibody. Unusual or pronounced vibration of the centrifuge must be eliminated to avoid false readings.

In every antigen-antibody reaction there is an optimum proportion of antigen to antibody. When this optimum condition exists, complete and clearly visible reactions result. Weak or negative reactions occur when there is a significant excess of either antigen or antibody. In undiluted serum, the antibody content may be so great with respect to antigen that no clear-cut reaction can be seen. Occasionally, such a reaction is erroneously called *negative*. The true nature of the false-negative reaction is revealed when the serum is diluted. When a visible reaction fails to appear because of antibody excess, we refer to this as the *prozone* phenomenon. When the quantity of antigen greatly exceeds the quantity of antibody, we also see a false-negative or weak reaction. We call this a *postzone* phenomenon. You can correct the quantitative relationship between antigen and antibody by serially diluting the antigen or antibody mixture used in the test.

False Agglutination and Umbilical Cord Serum. Some cord sera produce agglutination that simulates true agglutination. Wharton's jelly from the umbilical cord is responsible for the phenomenon.

Hemolysis. Hemolysis is often overlooked as evidence of antigen-antibody interaction. A poorly trained technician may have no reservations about using serum that shows evidence of hemolysis. Yet, if further hemolysis occurs because of antigen-antibody activity, the effect is masked and can be overlooked. While many other factors play a part, you should remember that hemolysis can result from antigen-antibody combination. In fact, some antibodies (for example, anti-Lewis) tend to show hemolysis rather than agglutination.

Exercises (207):
Match each term in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

____ 1. These reactions usually interfere with the invasion of the foreign antigens and protect against disease.
____ 2. Antigen-antibody reactions can be best detected and studied in the laboratory through these types of reactions.
____ 3. May cause disease such as acquired hemolytic anemia or idiopathic thrombocytopenic purpura.
____ 4. Rouleaux formation, false agglutination in which cells stack together.
____ 5. Granular agglutination and less regular.
____ 6. May result from abnormal proteins or factors resulting from medication.
____ 7. Does not occur as a rule when fresh, sterile sera are used.
____ 8. Can occur in certain patients with sepsis.
____ 9. In vivo infection of such may so alter the cells causing them to be capable of being agglutinable by many sera.
____ 10. Nonspecific clumping of an individual's cells by factors in his own plasma or serum.
____ 11. Most common at low temperature.
____ 12. Does not disappear after warming.
____ 13. Results if centrifugal force packs the cells too tightly.
____ 14. May cause red cells to become trapped and the particles have the appearance of agglutination.
____ 15. Is necessary to allow complete clumping when erythrocytes are mixed with very small amounts of specific antibody.
____ 16. When visible reaction fails to appear because of excess antibody.
____ 17. When the quantity of antigen greatly exceeds the quantity of antibody causing false-negative or weak reaction.
____ 18. Substance from the umbilical cord that is responsible for false agglutination phenomenon.
____ 19. A reaction caused by the anti-Lewis antibody and is often overlooked as evidence of antigen-antibody interaction.

Column B

a. Wharton's jelly.
b. Neutralization.
c. Prozone phenomenon.
d. Postzone phenomenon.
e. Adequate centrifugation.
f. Fibrin.
g. Pseudoagglutination.
h. Panagglutination.
i. Autoagglutination.
j. Polyagglutination.
k. *In vitro* reactions.
l. *In vivo* reactions.
m. True agglutination.
n. Hemolysis.

208. Define complement; cite its characteristics, its importance, and some antibodies that fix complement.

Complement. Complement is a thermolabile substance found in normal serum that is both necessary and responsible for the hemolysis of red cells in antigen-antibody reactions. Complement is believed not to increase during immunization. Various antibodies fix complement but some do not. Antibodies that fix complement include anti-A, anti-B, Anti-Le^a, anti-Le^b, anti Jk^b, anti-K, anti-Fy^a, and some others. However, antibodies of the Rh system do not usually fix complement. At temperatures lower than 37° C, complement-fixation occurs with cold *incomplete* anti-H. This can result in hemolysis of cells exposed to temperatures below 37° C. In normal serum, complement activity is maintained at a level adequate for detection of blood group antibodies for 3 months if the serums are stored at -90° to -55° C, for 2 weeks when stored at 4° C, for less then 36 hours when stored at room temperature (20° C to 24° C), and for only a few hours when the sera are maintained at 37° C. In other words, if any antibody reaction takes place, complement, if present, becomes attached by the antigen-antibody complex, but the quantity of complement is insufficient to cause cell lysis. The term "incomplete antibody" denotes an antibody that adheres to the surface of red cells suspended in saline but fails to agglutinate them. This term was more common in early blood banking literature. *In vivo* antibody coating and/or hemolysis may result from complement-fixation. The use of an enzyme in treating cell preparations may alter certain antigens, for example, M, N, and Fy^a, and thus alter complement-fixation properties so that test results are unpredictable.

We know that some antigen-antibody reactions are complement-dependent. This can be deomonstrated by studies of complement inactivation. It can also be easily shown in the laboratory that most human blood group antibodies do not hemolyze untreated red cells *in vitro*. When the hemolysis of untreated cells does occur, complement-fixation is a possibility. Actually, the importance of complement in blood-banking is best illustrated *in vivo* because survival times of erthrocytes correlate closely with the presence or absence of complement-binding antibodies. Cells coated with complement-fixing antibodies are removed from the circulation much faster than cells sensitized with non-complement binding antibodies.

Impairment of Complement. Significant impairment of complement activity may be observed with heparin. To restore lost complement activity fresh serum of the appropriate ABO group containing complement and free of irregular antibodies or soluble blood group substances (ABH, Lewis) may be added. Generally, one drop of fresh serum to two drops of stored serum is adequate. Other types of anticoagulant also have an antagonistic (anticomplementary) effect because they bind Ca++ and Mg++ ions, both of which are required cofactors in the complement sequence.

Complement binding antibodies may be IgM or three of the subclasses of IgG but not IgA. The participation of complement in blood group antigen-antibody reactions is demonstrated either by completion of the complement sequence to produce hemolysis or by the demonstration through the antiglobulin reaction that complement components have been bound to the red cell. Single molecules of IgM but two or more IgG in close proximity are required to bind complement. The finding that red cells can be recovered from the circulation containing complement components clearly indicates that in some cases *in vivo* activation of the complement sequence does not necessarily produce hemolysis. Usually only anti-A, anti-B, anti-PP$_1$p^k(Tj^a), and anti-Le^a lyse normal red cells *in vitro* with any regularity and are referred to as *hemolysins*. Examples of complement fixing antibodies in addition to those above are anti-Le^b, anti-P anti-Vel, anti-Jk^a, anti-Jk^b, anti-Fy^a, anti-K, anti-S, and anti-I. Not all antibodies of these specificities fix complement, and those that do fix complement do not necessarily produce *in vitro* hemolysis. Cells coated with complement can be effectively detected by an antiglobulin serum with activity against β-globulins.

Exercises (208):

1. What is complement?

2. What are some antibodies that fix complement?

3. Which antibodies do not fix complement?

4. At temperatures lower than 37° C, with what antibody does complement-fixation occur?

5. What can happen to the cells at temperatures lower than 37° C when complement-fixatio occurs with cold "incomplete" anti-H?

6. To what does the term "incomplete antibody" refer?

7. What antigens may be altered by the use of enzymes and how?

8. With what conditions are the survival times of RCBs closely correlated?

9. Significant impairment of complement activity may be observed with what given anticoagulant?

10. How may other types of anticoagulants have an anticomplementary effect?

11. All of the following antibodies are complement binding (IgM, IgG_1, IgG_2, IgG_3, IgA) except which antibody?

12. Which antibodies lyse normal red cells and are referred to as *hemolysins*?

209. Cite some necessary conditions upon which optimum antibody reactions are dependent.

Conditions of Reaction. The reaction between antigen on the red cell surface and antibody is reversible so that the quantity of cell bound antibody at equilibrium will vary depending on the reaction conditions and the equilibrium constant of the antibody. The reaction conditions should be designed to maximize the quantity of cell bound antibody at equilibrium, to facilitate detection of either blood group antigen or antibody. Some of these reaction conditions are described below.

Temperature. Most blood group antibodies show their greatest reactivity over a restricted temperature range, some at 4° C, others at approximately 22° or at 37° C. Antibodies reacting best at lower temperatures have been described as *cold* antibodies and those active at 37° C as *warm* antibodies. Agglutinins having maximum reactivity at one temperature may have sufficient thermal amplitude to be active at others. Antibody activity is usually tested at room temperature and at 37° C. Antibodies active at 37° C are considered most important clinically although *cold* antibodies may be of importance in patients subjected to hypothermia. You should remember that reactions that appear stronger at room temperature than at 37° C are commonly even better in the cold, but the widespread occurrence of *nonspecific* cold auto-agglutinins makes it more practical to detect some cold-agglutinating antibodies, for example, anti-A, anti-Lewis, anti-M, and anti-P_1, at room temperature. This allows separation and identification of cold-acting antibodies with a broader thermal range of reactivity.

pH. The pH optimums for antibody reactivity in most blood group systems have not been investigated. For anti-Rh_o(D) (an IgG immunoglobulin) the optimum pH lies between 6.5 and 7. Antibodies of other blood group specificities may have a different pH optimum. Some examples of anti-M (usually an IgM immunoglobulin) react best at pH 5.5. At values above 8.0 or below 6.0 there is usually loss of reactivity.

Incubation time. Time is required for the antibody—red cell reaction to reach equilibrium. The amount of time required to reach this state will depend upon other variables. The rate of antibody binding is greatest initially, so incubation times for routine laboratory procedures may be relatively short. It is important to standardize the incubation time. In one Rh system that has been studied, uptake of antibody is rapid, 80 percent within 10 minutes, and reaches equilibrium within an hour. Reactions between anti-A and A_1 cells are even faster, but reactions with weaker subgroups of A take much longer to reach equilibrium.

Exercises (209):

1. Antibodies considered to be most important clinically are active at what temperature?

2. List some cold agglutinating antibodies which may be detected at room temperature.

3. What is the optimum pH reactivity for anti-Rh_o(D)?

4. Anti-M reacts best at what pH?

5. Incubation times for routine laboratory procedures may be relatively short. Why?

210. Define ionic strength and zeta potential and point out the role they play in antibody reaction involving red cells.

Ionic Strength. The ionic strength of the reaction medium is one of the physiocochemical conditions that plays an important role in the binding of antibody to red cell antigens. Ionic strength is a measure of intensity of the electrical field due to ions in solution. Electrostatic forces (interaction of positive and negative charges) play an important role in antibody reaction involving red cells. Red cells carry a large electronegative charge which serves to keep red cells from spontaneously aggregating and thus enables them to function efficiently in oxygen transport by maintaining a large surface area for diffusion. If red cells and antibody are incubated in a medium of low ionic strength, that is, low concentration of dissolved salts, the amount of antibody bound to the cells will increase. Thus for routine work, the commonly used 0.85 to 0.9 per saline solutions are advised. If the ionic strength is too low, autoagglutination and false positive results will occur. Thus, work with low-ionic strength solutions must be carefully controlled.

Zeta Potential. When red cells are suspended in an electrolyte solution the cations (positive) are attracted to the negatively charged red cells and the red cell becomes surrounded by a diffuse double layer of *ionic cloud* which travels with the red cell as if it were part of the cell.

The negative charge of red cells results from the ionization of carboxyl groups of N-acetyl neuraminic acid residues (NANA) on the red cell membrane.

The outer edge of this RBC layer is called the *surface of shear* or the slipping plane. The effective charge (potential) of the red cell, called the zeta potential, is determined at this plane and causes the electrostatic repulsion between one red cell and another. For agglutination to take place, a force must be exerted to overcome this repulsion, or the antibody molecule must be long enough to bridge the distance.

In the first stage of agglutination, reducing the ionic strength of the medium decreased the electropositive cloud of cations surrounding the red cells and facilitates the interaction of electropositive IgG with the negatively charged red cell. This adsorption of antibody to the red cell reduces the electronegative charge of the red cell and reduces the zeta potential, thereby accelerating the second stage. Studies with iodine-labelled IgG and anti-Rh_o(D) clearly show that both the quantity of antibody bound to the cell and the rate of binding is markedly increased as the ionic strength of the reaction medium is decreased.

It is important to recognize that electrostatic forces may produce different effects on the two stages of the agglutination reaction. Increasing the ionic strength of the medium affects the second stage by decreasing the zeta potential so that the net repulsion distance between red cells is decreased. However, the ability of the antibody to bind is hindered, so no net increase in agglutination may be seen.

Enzyme modification reduces the net negative charge of the cell through the loss of sialic acid residues, which results in a reduction of the intensity of the electropositive ionic cloud around the cell, reducing the zeta potential and enabling the electropositive antibody (if the pH is appropriate) to approach more readily and interact with the red cell. Reducing ionic strength produces a similar effect, but in this case the net negative charge of the red cell is not reduced; rather, there are insufficient positive ions in the solution to form the electropositive ionic cloud around the red cell.

Increasing the dielectric constant of the medium by the addition of various colloids (for example, albumin) increases the charge dissipation characteristics of the medium and thus reduces the electrostatic repulsion between cells.

Exercises (210):

1. What is ionic strength?
2. What specific role does the ionic strength of the reaction medium play?
3. What will happen to the antibody bound to the cells if incubated in a medium of low ionic strength?
4. What result will occur if the ionic strength of the medium is too low?
5. What is zeta potential?
6. From what does the negative charge of red cell result?
7. In the first stage of agglutination when the ionic strength of the medium is reduced, what happens to the electropositive cloud of cations surrounding the red cells? What action follows?
8. What effect does the adsorption of antibody to the red cell have on the electronegative charge and the zeta potential?
9. What effect can increasing the ionic strength have on the zeta potential?
10. How does enzyme modification reduce the net negative charge of the cell?
11. How does the addition of albumin to the medium reduce the electrostatic repulsion between cells?

1-2. Rh Isoimmunization

Rh isoimmunization is of special concern to the clinical laboratory because it is the most common cause of hemolytic disease of the newborn (HDN), referred to in earlier literature as erythroblastosis fetalis. This fact has always involved the laboratory of hospitals with an obstetric service.

Rh typing and antibody studies are a necessary part of the workup in known and suspected cases of HDN, both during pregnancy and after birth. The direct Coombs procedure and its relevance to this problem are well known to any laboratory technician who has performed the Coombs test on cord blood at all hours of the day and night. The blood bank technician who secures and prepares blood for exchange transfusions is also keenly aware of the impact the so-called Rh problem has on the technician. The advent and use of anti-Rh$_o$(D) immune globulin has been spectacularly successful in preventing Rh immunication and consequently Rh erythroblastics fetalis (HDN), (EBF). We will discuss the use of anti-Rh$_o$(D) immune globulin (Rh$_o$ Gam)^R later in this section.

211. Identify the characteristics of hemolytic disease of the newborn (HDN) and the related mechanism of maternal immunization.

Characteristics of HDN. Hemolytic disease of the newborn is characterized by the destruction of an infant's red blood cells by specific antibodies, which were transferred across the placenta from the mother. The disease actually begins *in utero* and may result in the death of the fetus. Often it is not a serious threat to the well-being of the infant until shortly after birth. As red cells are destroyed, the infant becomes jaundiced, and the oxygen-carrying capacity of the erythrocytes that remain functional may become inadequate. In this case the infant suffers brain damage and possible death.

Mechanism of Maternal Immunization. The production of antibodies by the mother may be in response to almost any antigen. However, the Rh antigens most often responsible for isoimmunization in pregnancy are D and c. ABO incompatibility can also present a problem. The immunoglobulin, identified as IgG or (γG-7S), is transferred to the fetus via the placenta. This means that the antigens which are not IgG (for example, anti-M, N, P$_1$, Le^a, and Le^b) rarely, if ever, cause hemolytic disease of the newborn. Previous pregnancies and blood transfusions possessing the same *foreign* antigen vastly increase the chances for a significant antibody titer in later pregnancies.

Exercises (211):

1. What condition causes destruction of an infant's red cells in HDN?

2. When the infant's red cells are destroyed, basically what is commonly observed and how is the oxygen-carrying capacity of erythrocytes affected?

3. What are the Rh antigens most responsible for isoimmunization in pregnancy?

4. What immunoglobulin has been transferred to the fetus via the placenta?

5. Why will anti-M, N, P$_1$, Le^a and Le^b rarely cause HDN?

212. State the purposes of the exchange transfusion and rules governing the choice of blood.

Purpose of Exchange Transfusion. The principal purpose of the exchange transfusion is to remove the body's coated cells and to replace them with other red cells that cannot react with the passively acquired antibodies.

You can readily see from your knowledge of genetic inheritance how a D negative mother and a D positive father can produce D positive offspring. It should also be apparent to you at this point why table 1-1, discussed earlier, is of importance to the practical as well as to the theoretical blood banker.

If hemolytic disease of the newborn develops, the only effective treatment includes an exchange transfusion. The purposes of an exchange transfusion are to:

a. Replace destroyed erythrocytes.

b. Remove antibodies to the infant's red blood cells.

c. Remove some small amount of bilirubin, but mainly to prevent a further rise in bilirubinemia. (In severely affected infants, the large amounts of fixed bilirubin present in tissues, aside from circulating nonfixed bilirubin, cannot be markedly influenced by exchange transfusion.)

It is interesting to note that serum albumin has an affinity for binding bilirubin and is, therefore, of therapeutic value. Though still at the research level, only the so-called nonfixed bilirubin is believed to have a toxic action on brain cells. If this is the case, it has been suggested that albumin-binding bilirubin rather than direct and indirect serum bilirubin be used as an index of the requirement for transfusion.

Additional Facts About Exchange Transfusion. Physicians perform exchange transfusions by alternately withdrawing blood from the infant and replacing it with an equal amount of fresh donor blood. This is done in 10 to 20-cc increments until 300 to 600 cc of blood is replaced, depending on the size of the infant. The exchange is usually carried out through the umbilical vein, though other vessels may be used. In selecting the type of blood for exchange transfusion, the most important consideration is that the fetus produces little or no natural antibodies; hence, most of the circulating antibodies present at birth are from the mother. Accordingly, any blood administered must be compatible with the mother's serum. Recently published articles emphasize the fact that although exchange transfusion deaths occur more frequently in cases of severe hemolytic disease, the mortality rate in full-term infants is negligible. The overall risk is 1 to 2 percent.

The cardinal rules governing the choice of blood for exchange transfusion are:

- Avoid the antigen responsible for the antibodies present in the mother's serum.
- Administer blood with phenotype specific for the mother as to Rh, Kell, etc.
- Use group specific cells compatible with the mother and child or group O with low-titer serum.
- Use fresh blood, preferably under 24 hours old, and never more than 5 days old.

When the mother and infant are of compatible blood groups, for example, mother and infant are both group A, then group A negative blood is preferred by most clinicians. In other cases, for example, the mother is group A and infant is group B, it is desirable to use low-titer group O negative blood. By *low-titer*, we usually mean a titer of anti-A and anti-B less than 1:50. Remember that blood selected for exchange transfusion *must be compatible with that of the mother.*

Exercises (212):

1. What is the principle purpose of the exchange transfusion?

2. What are the two given primary benefits of an exchange transfusion?

3. Why must blood used for an exchange transfusion on an infant be compatible with the mother's serum.

4. How fresh should the blood be for exchange transfusion?

5. In an ABO incompatibility, if the mother is group A and the infant is B, what blood is recommended for an exchange?

6. What does "low-titer" mean?

213. Cite the purpose of Rh (D), human immune globulin, selection criteria for candidates, purpose of crossmatch, and time of administration.

Purpose of Rh (D) Immune Globulin (Human)-RhIG. Since its discovery, experience has shown that routine administration of RhIG to unsensitized Rh negative mothers who deliver Rh positive babies is an effective means of preventing Rh alloimmunization. The prefix *allo-* is often used by modern investigators in place of *iso-*. Researchers utilized the principle that complete suppression of active immunity can be caused by administering a passive antibody. Thus, passive antibody can cause specific immunosuppression of the active immunity that follows injection of an antigen.

The blood bank plays an essential role in the selection of candidates because accurate blood grouping and antibody detection are absolutely necessary.

Selection of Candidates. Candidates for Rh immune globulin are patients who are at immediate risk of immunization. Candidates for this prophylaxis are mothers who are Rh negative and D^u negative, have no detectable anti-Rh_o (D) antibody, and have an Rh positive or a D^u positive newborn.

In addition, all Rh negative women who have abortions are candidates unless the father or fetus is known to be Rh negative.

Evidence exists that a small percentage of women become isoimmunized at the time of spontaneous abortion. It is also possible for isoimmunization to occur during an ectopic pregnancy. Rh_oGAM is often administered to patients with abortion or ectopic pregnancy if they otherwise satisfy the criteria listed previously, except that blood studies cannot be performed on the fetus. As previously indicated, it must be assumed that the fetus is Rh_o positive or D^u positive, unless the father of the fetus is known to be Rh negative.

The following women are *not* candidates:

a. Rh_o negative women whose serum contains anti-Rh_o(D). (Rh negative women whose serum contains other unexpected antibodies are candidates.)

b. Rh_o negative women who deliver Rh negative babies.

c. Rh_o positive or D^u positive women.

When the direct Coombs test is performed on the infant's red cells, it must be definitely known that the positive Coombs test is *not* due to Rh isoimmunization.

ABO differences between the mother and infant can cause a positive Coombs—this should not be considered an absolute contraindication to the use of Rh_oGAM. Other causes of positive Coombs are common, and, unless due to the presence of anti-Rh_o, are not pertinent. The mother's serum may give positive Coombs reactions from causes other than the presence of circulating anti-Rh_o. When a question exists regarding the cause of a positive antibody screening test, the presence or absence of anti-Rh_o should be confirmed by tests with a panel of known cell types.

Legal liability is possible if isoimmunization develops in the mother as a result of failure to administer Rh$_o$GAM in the absence of contraindications that may arise from patient or laboratory considerations. This product is not to be administered to the infant.

Crossmatching. The question has arisen to whether a crossmatch is really necessary before administering Rh immune globulin. In such cases of an enormous quantity of fetal bleeding or transfusion of a large amount of incompatible blood, one cannot expect the crossmatch to be compatible; yet the product should be given. Cases have been reported where Rh immune globulin has been administered without harm to Rh-positive persons. Nevertheless, an incompatibile minor crossmatch serves to prevent accidents due to mistakes in identification, and it can provide warning that a large number of Rh-positive red cells have entered the recipient's circulation.

Time of Administration. RhIG is supplied as a sterile clear injectable for intramuscular administration into the mother within 72 hours after delivery if she meets the above criteria. It is a highly concentrated solution of IgG and anti-Rh$_o$(D), free of hepatitis virus, and derived from human plasma. In the case of a mismatched transfusion, treatment should be started as soon as the problem is detected.

The amount of RhIG supplied in one container is generally in excess. It is sufficient if the fetomaternal bleed is 30 ml or less. The entire contents of the container should be injected since the volume of Rh positive fetal cells which escapes into the maternal circulation is an unknown.

Rh$_o$GAM should be stored under the same conditions used for whole blood; that is, 4° to 6° C under controlled conditions, with maintenance of a continuously recording thermometer. A proper blood bank refrigerator should be available for any facility performing crossmatching. The storage period depends on this control. Freezing is prohibited. Detailed information on instructions for use, control, and procurement, as well as laboratory guidance regarding this pharmaceutical, is provided by the manufacturer.

Exercises (213):

1. For what purpose is the Rh$_o$ human immune globulin given?

2. The use of RhIG is based upon what principle?

3. What does the prefix *allo-* mean?

4. What basic criteria must candidates for Rh$_o$GAM meet?

5. When is the Rh$_o$GAM not administered to Rh negative women who have abortions or extopic pregnancies?

6. Give three situations in which patients may be excluded as candidates for Rh$_o$GAM.

7. If the Coombs is positive due to ABO incompatibility or circulating antibodies other than anti-Rh$_o$, should Rh$_o$GAM be administered? Why?

8. How should the presence or absence of anti-Rh$_o$ be confirmed?

9. The incompatible minor crossmatch provides what two essential items of information?

10. Within what maximum time after delivery should Rh$_o$GAM be administered?

11. Why should the entire contents of the Rh$_o$GAM be injected?

12. At what temperature should Rh$_o$ human immune globulin be stored?

Blood Group Systems

THE ABO system was neither established nor suspected until 1900 when Karl Landsteiner, through his research, observed that there were differences among the bloods of humans. After a considerable amount of research, Landsteiner and his assistants were able to categorize blood into three groups, A, B, and O. A year later, von Decastello and Sturli found the fourth group which they called AB.

In 1940, Landsteiner and another American, Alexander S. Wiener, discovered the RH factor through experiments with rabbits and the Rhesus monkey. This led to further work in the development of the RH system, which was found to be independent of the ABO system of classification. A year after Landsteiner's discovery, Philip Levine, together with Burnham, Katzin, and Vogel, identified the Rh factor clinically in a case of hemolytic disease of the newborn. At the same time the Rh factor was discovered independently by Paul Moreau, who worked in Occupied Belgium.

In this chapter, we will explore some of the modern concepts associated with blood groups as they are currently identified.

2-1. The ABO and Lewis Groups

At this point in your career, you are quite familiar with the ABO blood group system, which Landsteiner discovered. Perhaps you are less familiar with the Lewis system discovered in 1946 by Mourant. We will consider both of these systems in this section because the chemistry of Lewis and of ABO blood group substances is similar.

214. Cite some characteristics related to the origin and development of ABO blood group antibodies and define natural and immune forms of blood group antibodies.

ABO System—General. From our discussion in Chapter 1, it is quite evident that there are antigens labeled A and B located on the red blood cells. If both antigens are present, the person is in blood group AB; and if neither antigen is present, he is in group O. It is also elementary that people possess antibodies in their serum for the antigen that is lacking on their red blood cells, as enumerated in table 2-1.

Development of ABO Antibodies. Antibodies are the result of antigenic stimulation, and it is the antigens, not the antibodies, that are under direct genetic control. "Naturally occurring" means that such antibodies are produced naturally, without injection or pregnancy, by natural exposure of the body to substances with antigenic properties identical, for all practical purposes, to those of the A and B antigens.

The approximate frequencies of the various blood groups in the United States is given in table 2-2. Group O is certainly the most common, and blood of this group is sometimes called the *universal donor blood* because of the absence of A and B antigens on the red cells.

Immune and Natural Antibodies. Antibodies are classified in various ways. We can classify them as (a) immune and (b) natural, or naturally occurring. Anti-A and anti-B antibodies can be immune as well as natural. By "natural" we mean that antibodies so described are normally found in a person shortly after birth and throughout adult life, and are not introduced by injections or pregnancy. It is theorized that ABO antigens are introduced into the body in food and in the air we breathe. Hence, exposure to ABO-type antigens occurs continuously from birth; but it is not until several weeks after birth that anti-A and anti-B titers in the infant are high enough to be detected by the usual technique of proof grouping. This explains why a newborn infant may proof group (back type) as group AB. Naturally occurring antibodies are IgM and immune antibodies are IgG antibodies.

Newborn infants are incapable of synthesizing IgG antibodies and are able to synthesize only reduced quantities of IgM. The majority of anti-A and anti-B present in cord serum is IgG and is passively acquired from the mother. The IgM anti-A and anti-B synthesized by the infant is demonstrable in occasional cord sera. Anti-A and anti-B that have been synthesized by the infant is demonstrable in most infants by 3 to 6 months of age. The titers of anti-A and anti-B are highest at ages 5 to 10 years and eventually decrease gradually.

Exercises (214):

1. What do we mean when we say ABO antibodies are "naturally occurring"?

TABLE 2-1
"NATURALLY OCCURRING" ANTIGENS AND ANTIBODIES OF THE ABO BLOOD GROUPS

Blood Groups	Antigen on the Erythrocyte	Antibodies in the Serum
A	A	Anti - B
B	B	Anti - A
AB	Both A and B	Neither Anti-A nor Anti - B
O	Neither A nor B	Both A and B

2. What stimulates the production of ABO antibodies?

3. What are immune antibodies?

4. How are naturally occurring antibodies produced?

5. What type of immunoglobulin antibodies are naturally occurring antibodies?

6. The majority of the anti-A and anti-B present in cord serum is what kind of immunoglobulin antibody?

7. Since reduced quantities of IgM are synthesized by the newborn infants and are occasionally demonstrable in cord sera, this may account for the difficulty in normally detecting what two antibodies?

8. At what ages are the anti-A and anti-B highest?

215. Point out the basic origin of the ABO antigens in terms of the characteristics and properties of the precursor substance.

Development of ABO Antigens. The antigens of the ABO system are well developed in the fetus but do not attain full antigenic strength until several months after birth. Geneticists have presented a theory regarding the genetic pathway leading to the production of the A, B, and H antigens on the red cells. In figure 2-1, you will note accordingly, that A and B antigens develop from H substance.

TABLE 2-2
FREQUENCIES (PERCENT) OF ABO GROUPS IN THE UNITED STATES

GROUP	WHITES	BLACKS	CHINESE
O	45	49	36
A	40	27	28
B	11	20	23
AB	4	4	13

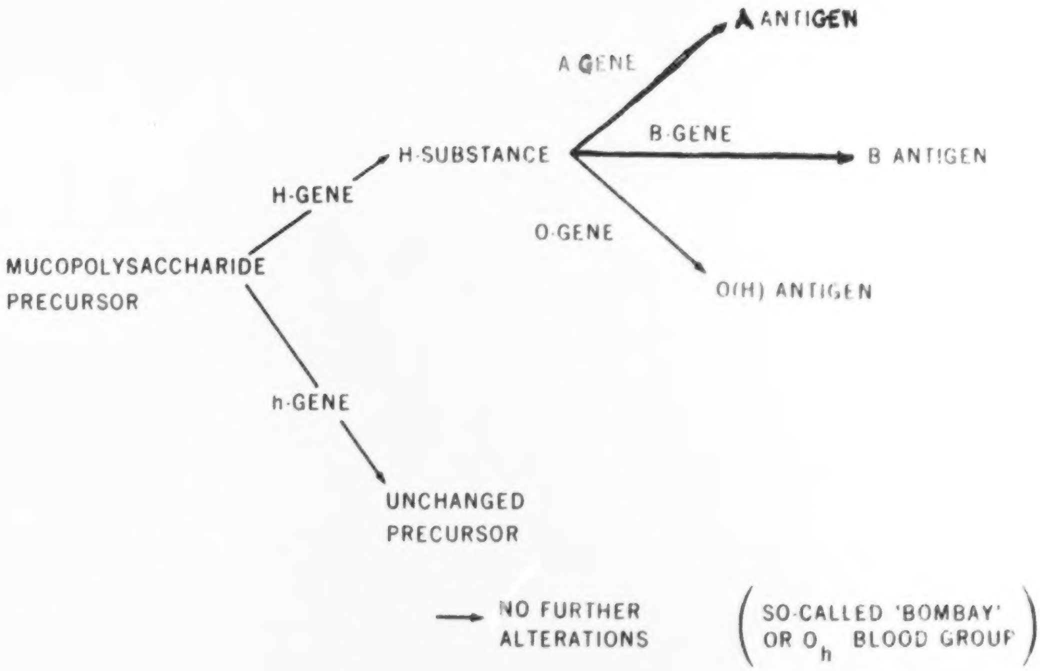


Figure 2-1. ABO antigen development.

This precursor is transformed into specific A or B substances under genetic influence. The O gene does not change the H substance; therefore, group O cells contain more H substance than cells of other groups. If the H substance is not transformed into either A or B, the red cell contains the H substance rather than the specific A or B antigen (or both). The H substance is not a product of the O gene, but a precursor of A and B antigens developed from a mucopolysaccharide. The O gene does not produce a recognizable effect, and because of this is sometimes referred to as an amorph. Blood groups other than O have lesser amounts of H substance and can have anti-H antibody. There is a rare kind of blood group O called O_h or Bombay blood group. (The term "Bombay" is now becoming obsolete). The blood of individuals in group O_h contains no H, A, or B substance. They have anti-H as well as anti-A and anti-B in their serum.

The A, B, and H antigens are present not only on red cells but also in body tissues and secretions. The presence of water soluble antigens in secretions is determined by the presence of a secretor gene, Se. Its allele se is recessive, so individuals homozygous or heterozygous for Se will be termed "secretors." Those possessing the genotype se/se are *nonsecretors.*

It may surprise you to learn that A and B antigens are not only present on the red cells, but have been shown to exist in varying concentration in most of the other body tissues with the exception of the brain and the spinal cord. Such discoveries have proved to be very useful to those immunochemists interested in studying the chemical structure of the A, B, and H determinants. A practical application of this phenomenon is in rape cases where seminal fluid is compared with semen from the suspect. The rationale of testing is based on the fact that A and B substances are secreted in the body fluids of most individuals.

Exercises (215):

1. What is the precursor substance that, under genetic stimulation, produces A or B antigens?

2. Why do group O cells contain more H substance than A or B cells?

3. What kind of substance is H substance?

4. What is unusual about the rare O_h (Bombay) blood?

5. In what other substances are A, B, and H antigens present?

6. What gene controls the secretion of A and B substances into the body fluids?

7. Those possessing the genotype Se/se are called __________.

8. The A and B antigens are present in most of the body tissues except what two organs?

216. Indicate whether given statements correctly reflect the characteristics and properties of the subgroups of A and B.

Subgroups of A. Classification of weak subgroups of A is based on: the reactivity of red cells with anti-A, anti-A,B, anti-H and anti-A, *(Dolichos biflorus)*; the presence or absence of anti-A_1 in the serum; and the secretion of A and H substance by secretors.

Variants of the A antigen. Variants of antigens are also commonly known. For example, there is more than one type of A antigen. The different variants of the A antigen are designated A_1, A_2, A_3, A_m, and A_x. Variants A_3, A_m, and A_x are relatively uncommon. Studies of group B and group O persons show that two distinct antibodies are common, namely anti-A_1, and anti-A_2. A person of group A_2 may have anti-A_1. If a patient with anti-A_1 in his plasma is given group A_1 blood, there may be a transfusion reaction. A distinction can be made between A cells and those of the weaker subgroups of A by the use of an absorbed anti-A (anti-A_1) serum. The absorbed anti-A (anti-A_1) serum will not react with cells of subgroups A_2 and A_2B, or weaker. The amount of A antigen on the red cells of the various subgroups decreases without a sharp dividing line between any two types. These antigens weaker than A_2 have been recognized as A_3, A_4, A_5, A_0, A_m, A_x, A_z, A_g. One authority suggests that A_x be used for A_4, A_5, A_0, and A_z. In addition, A_m and A_g appear to have certain similarities.

Applications. The weak expressions of A should be recognized so that units from such donors are not used in emergency situations without adequate compatibility tests. When given to a group O recipient, such a unit was reported to have caused a transfusion reaction. Recognition of weakly reacting A antigens may be important in paternity testing. Weak expressions of A, particularly A_x, are better detected with anti-A, B (group O) serum than with the anti-A from group B persons. The presence of weak A antigen can be confirmed by absorption and elution studies.

Subgroups of B. Subgroups of B, which are infrequent, usually have decreased but detectable amounts of B antigen. These antigens are designated B_1, B_2 and B_3. Other forms such as B_x, B_w, and B_m have also been described.

Exercises (216):

Indicate whether each of the following sentences is true (T) or false (F). If you indicate false, explain your answer.

T F 1. A and H substances by secretors may be used for classification of weak subgroups of A.

T F 2. The presence or absence of anti-B_1 may be used for classification of weak subgroups of A.

T F 3. A person of group A_2 may have anti-A_2.

T F 4. If a patient with anti-A_1 in his plasma is given group A_1 blood, no harm will result to the patient.

T F 5. Absorbed anti-A (anti-A_1) serum is used to distinguish between anti-A, and other weaker subgroups of A.

T F 6. The absorbed anti-A (anti-A_1) will also react with the cells of subgroups A_2 and A_2B or weaker.

T F 7. Recognition of weakly reacting A antigens may be important in paternity testing.

T F 8. Weak expressions of A, particularly A_x, are better detected with anti-A, -B (group O) serum than with the anti-A from group B persons.

T F 9. The presence of weak A antigen can be confirmed by adsorption and dilution studies.

T F 10. The subgroups of B are more frequent, and have increased detectable amounts of B antigens.

217. State the method of blood grouping for transfusion blood, sources of error for ABO grouping, and the reason for performing reverse grouping on all donors and recipients.

ABO Cell Grouping. Because of the permanent, reliable characteristics of blood group antigens and the simplicity of technique, blood groups are routinely identified by direct cell grouping. This can be done on a microscope slide or in a tube. Generally speaking, tube methods are more reliable. You must be careful to follow the instructions furnished with the grouping or typing antiserum you are using. Correctly used, the term "typing" is limited to identification of Rh antigens, whereas *grouping* applies to other antigen systems. Some antisera are used with saline-suspended cells, while other antisera must be used with a serum or protein suspension. Reasons for this will be clearer as you study later in this volume the necessity of a high protein medium. Slide methods are recommended only for screening procedures or identification for administrative purposes, for example, to be used on ID cards.

Blood for transfusion should always be tested by the tube method. There are many possible errors in *forward* (direct) *grouping*. Antisera are sensitive biologicals that can become contaminated, impotent, or otherwise unsuitable. The technician may be confused by misleading reactions due to cold autoagglutinins, saline reactive agglutinins other than A and B, and polyagglutinable erythrocytes. Reactions caused by subgroups, clerical mistakes, haste in reading, and reading over a warmed surface, are also possible causes of error in direct cell grouping.

Reverse Grouping. To supplement forward grouping, serum grouping is an essential quality control procedure. It is also called *proof grouping, reverse grouping,* or *back grouping*. The procedure for serum grouping is found in AFM 160-50. The American Association of Blood Banks stresses that serum grouping should be performed on all recipients and donors. Infants less than a few months of age may not have developed sufficient anti-A and anti-B to proof-group correctly.

By the use of known A and B cells, serum grouping reactions can be interpreted as outlined in table 2-3. There are good reasons for using A_2 and O cells as well as the A_1 cells recommended in AFM 160-50. The reason for using A_2 cells is to prevent misidentification of A_2 as group O. (A_2 and A_2B persons may have anti-A_1 in their sera.) Ordinarily, this possible discrepancy would be brought out in front grouping because most anti-A grouping serum detects A_2 as well as A_1. We are suggesting, however, that in some cases the use of A_1 cells by themselves could *confirm* a subgroup of A erroneously forward-grouped as O. This would be a double error.

The use of group O cells in serum grouping provides a control measure. If agglutination is due to a cold agglutinin, the group O cells would also agglutinate. Prepared suspensions of cells for serum grouping are available commercially. If you prepare your own cell suspensions, you should take extra care in proper group identification. Red blood cells suspended i saline may undergo rapid deterioration. It is essentia then, that you use the cell suspensions as soon as possible after preparation and certainly no later than the day of preparation.

Exercises (217):

1. Why should blood grouping tests on transfusion blood be performed by the tube method?
2. List some sources of error for ABO grouping.
3. What other names mean the same as serum grouping?
4. Why must we perform serum grouping tests on all donors and recipients?
5. Which blood group is most often mistyped?
6. What procedure will usually eliminate the mistyping error mentioned in question 5?
7. If commercially prepared test cells are not available, how often should the tests cells be made and why?
8. Why are group O cells used in the serum grouping procedure?

218. Indicate whether given statements correctly reflect the nature and characteristics of the Lewis blood system.

Lewis Groups. The Lewis blood system has been of particular interest to immunologists because of its relationship to the salivary secretion of blood group specific substances. The anti-Lewis agglutinins designated anti-Le^a and anti-Le^b occur naturally but quite infrequently in human serum. These antibodies, when

TABLE 2-3
INTERPRETATION OF SERUM GROUPING REACTIONS
(Group O cells should be used as a control)

Blood Group	A Cells	B Cells
A	No agglutination	Agglutination
B	Agglutination	No agglutination
AB	No agglutination	No agglutination
O	Agglutination	Agglutination

present, are usually nonreactive at body temperature and exist in low titer. Transfusion reactions involving Le^a incompatibility have been reported.

Nature of the Lewis Antigens. In contrast to the usual blood groups, there is no agglutinogen in the Lewis system. Instead, there is a water-soluble Lewis substance present in body fluids and secretions such as saliva, semen, and vaginal fluid. Lewis substances are only secondarily adsorbed onto the red blood cells. They are a group of soluble polysaccharide antigens of body fluids. This adsorption accounts for the reaction of the red cells when mixed with anti-Lewis serum.

Lewis antigens are thought to occur in saliva and in the serum, from which they are adsorbed by the red blood cell. Nonsecretors have the phenotype Le(a+b–). Persons who inherit the Lewis gene but are secretors have the phenotype Le (a–b+). These phenotypes are referred to as Le^a and Le^b, respectively. The presence of A_1 may interfere with the expression of Le^b. Various involved relationships between the ABO system and the Lewis systems have been postulated. The erythrocytes of Le^b positive individuals are agglutinated by anti-Le^b serum. Cells of the A_1 subgroup, group B, or A_1B, agglutinate only with certain Le^b antisera. This leads to the conclusion that only group O blood has a significantly detectable amount of the Le^b antigen.

Cells of the Le^a group are presumably all agglutinated by Le^a antiserum. Further, Le^a positive red cells have no Le^b antigen. Reactions to Lewis antigens during the first 15 months of life change. Some people are negative for Le^a and for Le^b. About 70 percent of the population is Le^b positive, and about 25 percent are Le^a positive. The rest are negative for both Le^a and Le^b.

The anti-Le^a antibody is found occasionally in genetically Lewis-negative individuals. The presence of anti-Le^a can be demonstrated with commercially available Le^a positive cells. Anti-Le^b is found even less often than anti-Le^a. Lewis antibodies are ***naturally occurring***, because they are found in individuals who have received no known antigenic stimulation except, of course, through their natural environment.

Exercises (218):

Indicate whether each statement is true (T) or false (F). If you indicate false, explain your answer.

T F 1. Lewis antibodies are usually reactive at body temperature.

T F 2. Like the ABO System, the Lewis agglutinogens are located on the red cells.

T F 3. There is a water-soluble Lewis substance present in body fluids such as saliva, semen, and vaginal fluid.

T F 4. Lewis substances are secondarily adsorbed onto the red blood cells and react positively with anti-Lewis serum.

T F 5. Nonsecretors of the Lewis antigens have the phenotype Le (a–b+).

T F 6. The phenotype Le (a+b–) is referred to as Le^a.

T F 7. The red cells of Le^b positive individuals are not agglutinated by anti-Le^b serum.

T F 8. Le^b positive cells of the A_1 subgroup, group B, or A_1B agglutinate only with certain Le^b antisera.

T F 9. Cells of the Le^a groups are all agglutinated by Le^a antiserum.

T F 10. About 70 percent of the population is Le^a positive.

T F 11. Lewis antibodies are immune antibodies because they are found in individuals who have received no known antigenic stimulation.

219. Point out the importance of the Lewis factors and problems encountered in blood banking with Lewis antibodies.

Importance of the Lewis Factors. Primarily, the Lewis system is important in transfusions because individuals who lack the substance in their saliva are Lewis negative, and can become sensitized to the Lewis factor if transfused with Lewis-positive cells. Future transfusion with Lewis positive cells could result in serious and even fatal hemolytic transfusion reaction. Lewis antibodies have not been linked to hemolytic disease of the newborn since they are of the 19-S variety or IgM type and thus do not pass through the placenta. Another reason is that in newborn infants the Lewis substance is not yet on the red cells.

Blood Banking Problems—Lewis Antibodies. Lewis antibodies frequently cause difficulties in the blood bank and when present are almost always in persons of Le(a–b–) phenotype; the most troublesome probably is anti-$Le^a + Le^b$. Because of the higher frequency of the phenotype Le(a–b–) in blacks than in whites, compatible blood for patients with this antibody is more easily found by screening black donors.

Remember that Le^a and Le^b reactivity of cells may be diminished by washing the cells, so Lewis typing should always be carried out with controls.

Exercises (219):

1. Why is the Lewis system important in transfusions?

2. What type of reaction is most likely if a Lewis-negative individual who has been sensitized is later transfused with Lewis positive cells?

3. What two possible reasons are given as to why Lewis antibodies have not been linked to hemolytic disease of the newborn?

4. What Lewis antibody is considered the most troublesome in the blood bank?

5. Since problems are most frequently encountered with individuals of the phenotype, Le(a–b–), what ethnic group constitutes a more likely source of compatible donors and why?

6. Why should Lewis typing always be done with controls?

2-2. Rh and Other Systems

The subject of Rh blood groups has been one of great interest since Levine and Stetson described their now famous clinical case of HDN in 1941. We have already discussed Rh isoimmunization in Chapter 1. Let us now study the Rh system as a taxonomic grouping. In concluding this section, we will also identify some of the other blood group systems, including the Kell, Duffy, Kidd, MNS, and a few miscellaneous classifications.

220. Indicate whether given statements correctly describe the nomenclature of the Rh system and the characteristics of the D^u antigen.

The Rh System—Nomenclature. A basic group of six closely related antigens makes up the Rh system. Fisher and Race have designated these as D and d, C and c, and E and e. The corresponding antibodies were designated by Wiener as anti-Rh_o and anti-Hr_o, anti-rh′ and anti-hr′, and anti-rh″ and anti-hr″, to designate allelic genes in a way that is not completely comparable to the Fisher-Race system. Each factor, except d (Hr_o), has been identified by a reaction with its specific antibody, produced in individuals who have been immunized. The antibody whose formation is stimulated most frequently is anti-D (anti-Rh_o), which reacts with the erythrocytes from 85 percent of the white US population and, in so doing, reveals D (Rh_o), the originally described Rh antigen. The major factors of the Rh-Hr system and their corresponding CDE notations are shown as follows:

Wiener	*Fisher-Race*
Rh$_o$	D
rh'	C
rh"	E
Hr$_o$	d (nonexistent)
hr'	c
hr"	e

Contrary to earlier information, anti-d typing serum is not available, commercially or otherwise. The term "Rh positive" commonly refers to D (Rh$_o$) positive blood. This is a somewhat dangerous simplication of terminology. A blood donor, for example, is considered Rh negative by most authorities if he is negative for D and D^u.

The D^u (Rh$_o$ Variant) Antigen. The term D^u or Rh$_o$ variant refers to RBCs that react weakly or not at all with saline agglutinating anti-Rh$_o$ (D) but positively with antiglobulin techniques. These cells may also react by direct tests with slide or modified tube test serums, possessing a high protein base. Red cells that contain the D^u factor are not usually agglutinated by anti-Rh$_o$(D) serum. D^u bloods give positive reactions only after being exposed to anti-Rh$_o$(D) antibodies and then tested with antihuman serum by the indirect Coombs technique. Some anti-Rh$_o$(D), CD, or CDE antisera detect this antigen, but many otherwise potent antisera do not. For this reason, all D negative blood donors must be further tested by the indirect Coombs technique.

Test for Rh$_o$ Variant. The presence of the D$_u$ antigen can best be detected by incubating the red cell suspension with anti-Rh$_o$(D) followed by antiglobulin testing. More precisely, the test cells are incubated with anti-D at 37° C for 1 hour and washed three times with saline. Run Coombs positive and negative control cells to check the effectiveness of your Coombs serum. Keep in mind that Coombs positive blood will type positive for D^u and, therefore, this is not a valid test with blood that is Coombs positive for a reason other than presence of D^u. Coombs serum is added, and the cells are centrifuged and examined for agglutination or hemolysis.

The American Association of Blood Banks (AABB) requires that all blood banks seeking its accreditation perform the indirect Coombs test for D^u identification. AFM 160–50, *Technical Methods and Procedures of the AABB*, states that every donor blood found negative on direct tests with anti-Rh$_o$(D) must be tested for the D^u antigen and found negative, if it is to be labeled Rh-negative. The possibility of immunizing the Rh-negative patient makes it desirable to identify D^u bloods as Rh-positive.

According to one authority, D^u positive people do sometimes form anti-D. Though regarded as a rare occurrence, it would, therefore, seem unwise to give D positive blood to a D^u positive, D negative recipient. The D^u variant occurs more often in blacks than in whites.

Exercises (220):

Indicate whether each of the following statements is true (T) or false (F). If you indicate false, explain your answer.

T F 1. Fisher and Race have designated the Rh system as Rh$_o$, rh', rh" Hr$_o$, hr', hr".

T F 2. Each factor, except Hr$_o$ has been identified by a reaction with its specific antibody.

T F 3. In comparing the Weiner nomenclature to the Race-Fisher, hr' is equivalent to e and rh' is equivalent to E.

T F 4. Contrary to earlier information, anti-d typing serum is available.

T F 5. A blood donor is considered negative if he is negative CDE D^u positive.

T F 6. D^u bloods will give positive reactions after being exposed to anti-Rh$_o$(D) antibodies and tested with antihuman serum.

T F 7. Cells for D^u are incubated with anti-D at 37° C for 2 hours, washed three times with saline, and Coombs serum added.

T F 8. The possibility of immunizing the Rh-negative patient makes it desirable to identify the D^u bloods as Rh-positive.

T F 9. D^u positive people can also produce anti-Rh$_o$(D), and should be given Rh$_o$(D) negative, D^u negative blood.

T F 10. The D^u variant occurs more often in whites than blacks.

221. Indicate whether given statements correctly reflect some characteristics of the other Rh antigens, Rh deletions, and Rh_{null}.

Other Rh Antigens. Other Rh antigens besides those already mentioned are known. Included are the V antigen discovered in 1955, the G antigen described in 1958, and the LW (Landsteiner-Wiener) antigen shown in 1964 to be genetically independent of other Rh genes. According to a tentative genetic pathway proposed for the development of the LW and Rh antigens, a common basic precursor substance (substance 1) in the presence of a dominant gene X'r is activated into a second precursor substance (substance 2) which is acted upon by the LW and Rh genes to produce the LW and Rh antigens. Finally, it is worth pointing out that rare cases have been described in which the red cells were lacking in some or all of the Rh antigens. In the latter instance, action of a suppressor gene is suspected in the case of a parental genotype *normal* with respect to the Rh system.

V antigen. The V antigen has a higher frequency in the blacks than in other groups. Its corresponding antibody is found frequently in combination with anti-Rh_o(D). The blood factor V is designated as hr^v in the Wiener terminology. Anti-hr^v serum separates hr-positive red cells of blacks into two subgroups—hr^v negative and hr^v positive; for example, Rh_o proper and Rh_o^v.

G antigen. The G antigen is normally found in all persons who have the C or D antigen. The corresponding antibody reacts with all C or D positive cells and has caused much confusion in trying to determine how a cde/cde woman who already possesses an anti-D can build an apparent anti-C during a pregnancy when the father is known to be cDE/cde. The process of separating anti-G from anti-D and anti-C can be achieved by a complicated series of absorptions and elutions.

The LW antigen. The antigen called LW (after Landsteiner and Wiener) received recognition as the explanation for an example of an Rh-positive individual with apparent antiR-Rh_o(D), but its theoretical importance extends beyond this. The LW antigen is present on both Rh-positive and Rh-negative cells, but is much more strongly expressed in Rh-positives. The rare individuals whose normally Rh-positive cells lack LW can produce anti-LW. The cells of LW negative, Rh-positive individuals do not react with guinea-pig and rhesus monkey serum. The antibody Landsteiner and Wiener developed by injecting rhesus monkey cells into guinea pigs does not, as was originally assumed, have the same specificity as immune anti-Rh_o(D); their antibody has now been shown to be anti-LW rather than anti-Rh_o(D). The weaker expression of LW on adult Rh-negative rather than adult Rh-positive cells accounts for the *D-like* specificity of anti-LW, but a variety of absorption, agglutination, and immunization studies have shown the two antigens to be different.

Rh Deletions and Rh_{null}. Rare individuals have been discovered who lack one or all of the common Rh antigens. The cells of these individuals fail to react with antiserum for rh″(E) or hr″(e) or any variants a this site, or for rh′(C), hr′(c) or for any variants at tha site. The genotype of such "missing" antigents or Rh deletions is written with a bar in the place of the determinant, for example, - - - De/ - - - De, CD- - -/CD, or - - -D- - -/ - - -D- - - . The antigen determined by such genes reacts more strongly with anti-Rh_o(D) serums than the products of normal genes.

Some cells have been found which fail to react with any Rh antiserum at all. The term "Rh_{null}" or — — —/ — — — was applied to this extraordinary cell type. Apparently an amorphic gene exists for the Rh system which, in double dose, results in absence of Rh antigenic activity. A completely different gene appears to result in suppression of Rh activity so that heterozygotes have reduced Rh activity and homozygotes have no activity at all.

These conditions are considered to be rare. Nevertheless, the blood bank technician should be aware of such phenomenal occurrences.

Exercises (221):

Indicate whether each of the following statements is true (T) or false (F). If you indicate false, explain your answer.

T F 1. The corresponding antibody for the V antigen is frequently found in combination with anti Rh_o(D).

T F 2. The blood factor V is designated as hr″ in the Wiener terminology.

T F 3. The G antigen is normally found in all persons who have C or E antigens.

T F 4. The process of separating anti-G from anti-D and anti-C can be achieved by a complicated series of absorptions and elutions.

T F 5. The LW antigen is present on both Rh-positive and Rh-negative cells, but more strongly expressed in Rh-negatives.

T F 6. The weaker expression of LW on adult Rh-negative than adult Rh-positive cells accounts for the "D"-like specificity of anti-LW.

T F 7. The genotype of missing antigens on Rh deletion is written with an asterisk in the place of the determinant.

T F 8. The term "Rh_{null}" is applied to the cell type which fails to react with any Rh antiserum and is written as — — —/— — — .

222. Identify given blood group antigens, their systems, and other rare antigens in terms of their occurrence, characteristics, detection, and significance.

Other Blood Systems. Human erythrocytes possess many known antigens. Since antigens are molecular complexes of relatively small size, thousands of them may be present on the surface of a red cell. In addition to ABO and Rh, some of the other clinically important antigens include Kell, Duffy, and Kidd. Blood antigens such as M, N, S, s, k, P, Lutheran, and Lewis are important in the exact identification of individual blood specimens. There is little doubt that these lists will expand as research in the field of immunohematology progresses. Blood bank technicians should be familiar with the most important of these blood antigens. A brief discussion of their occurrence, makeup, and detection is presented in the paragraphs that follow.

Kell system. The Kell blood system includes the antigens K and k. The K antigen is present in approximately 9 percent of the population, and sensitization to this antigen has been an occasional cause of hemolytic transfusion reaction and hemolytic disease of the newborn. Inheritance of the Kell blood antigens is determined by two genes alleles, K and k. All individuals who are homozygous kk or who are heterozygous for K together constitute about 98.8 percent of the population. As a result of the rarity of homozygous KK individuals, sensitization to this factor is extremely uncommon. With the use of two sera, anti-K (Kell) and anti-k (cellano), it is feasible to determine an individual's specific Kell genotype. Anti-K shows a dose response that may be useful in the detection of heterozygous individuals. Anti-K is almost always of the incomplete variety of antibody and is usually most reactive in the indirect Coombs test. Examples of anti-Kell, which react exclusively with the enzyme method, have been reported. Other Kell variants have been described such as Penny(Kp^a), Rautenberg(Kp^b), Peltz(K^o), Sutter(Js^a), Matthews(Js^b), K^w, Cl(KL), Ul^a.

Duffy system. The two antigenic factors comprising this system are designated Fy^a and Fy^b. They are inherited through a pair of gene alleles also named Fy^a and Fy^b, respectively. Anti-Fy^b serum is quite rare and, as a result, the presence of Fy^a accounts for the two blood types ordinarily recognized in the Duffy system. The Duffy positive and negative types determined by the use of anti-Fy^a serum are referred to as Fy^a positive (a+) and Fy^a negative (a−). The Fy^a negative type occurs in about 35 percent of the Caucasian population; therefore, immunization against Fy^a is an occasional cause of hemolytic transfusion reaction. The anti-Fy^a antibody can sometimes be recognized only with the use of anithuman serum. The albumin or trypsin methods are, as a rule, quite inadequate.

Kidd system. The antigenic factors of the Kidd system are designated Jk^a and Jk^b. Kidd isoimmunization due to the Jk^b antigen is very infrequent, and most of the cases reported involve Jk^a. The blood bank technician is generally concerned with two blood types in the Kidd system. These are Jk^a positive and Jk^a negative, as determined by anti-Jk^a serum. Jk^a negative occurs in about 25 percent of the population, and sensitization to Jk^a positive cells occurs occasionally as a consequence of pregnancy. Antibodies of this system have caused delayed transfusion reactions when they have not been present in demonstrable levels at the time of transfusion.

Lutheran system. The antigens in the Lutheran blood system are designated Lu^a and Lu^b. These factors are not common in transfusion reactions. The incidence of the Lu^a type is about 8 percent. There is evidence that the Lutheran system may be linked with the previously mentioned Lewis system. Lu^b has been characterized as an IgA antibody which accounts for its failure to cross the placenta.

P system. The P system has been investigated and implicated in transfusion reactions. Recently discovered complexities in this system have prompted a change in its antigen nomenclature. The P positive cells of the old classification are now regarded as P_1 and P negative as P_2. These individuals have antibodies that react with both P_1 and P_2 cells. The P_1 antigen is present in 79 percent of Caucasians and 94 percent of Negroes. Anti-P_1 is present in two-thirds of the people with P_2 and is a naturally occurring antibody reacting at low temperatures.

The problem of selecting suitable blood for transfusion is usually compounded by one or more of the following facts:

a. The P_1 antigen varies considerably in strength on the cells of different P_1 positive persons.

b. The P_1 antigen deteriorates more rapidly than any other antigen on red cells. Frequently, only fresh cells can be reliably typed for P_1.

c. Cold temperatures enhance reaction; however, anti-I antibodies may obscure the P_1 pattern.

d. It is very difficult to adsorb anti-A and anti-B from sera containing anti-P_1 so that typing sera suitable for use with A and B bloods are difficult to prepare.

Although some patients with anti-P_1, active only at 22° C and below, have been transfused with P_1 positive blood with no evidence of red cell destruction, other examples of the antibody have caused transfusion reactions.

The rare individuals of phenotype p_1 previously called Tj(a–), have anti-PP_1^{Pk} (previously called anti-Tj^a) in their serum. This antibody frequently occurs as a hemolysin and has caused both hemolytic transfusion reactions and hemolytic disease of the newborn. The rare individuals of phenotype P^k have anti-P in their serum. Anti-P reacts with P_1 and P_2 cells but not usually with p cells. No anti-P_2 antibody exists: the P_2 phenotype is recognized as having cells that are P_1 negative, P positive.

Rare Antigens. There are certain blood antigens that have a very infrequent occurrence in random or unrelated blood specimens. These factors are, therefore, referred to as *private* or family antigens. These blood antigens include the following: Levay, Gr, Jobbins, Mi^a, Becker, Ven, Ca, Be^a, Wr^a, Di^a, By^a, Vw, and Chr^a.

In contrast to these private or family antigens, there are high incidence or *public* antigens. The latter were discovered through antibodies that react with the red cells of most of the population. Although these antibodies are rare, they can create great difficulties in crossmatching because of their capacity to react almost universally. Examples of public or high incidence antigens are: Vel(Ve^a), Cartwright(Yt^a), Gerbich(Ge), Auberger(Au^a), I-i, and Sm, Kp^b, and Js^b.

It is virtually impossible at any given point in time to be exactly certain how many different red cell antigens have been detected. Since research is being conducted the world over, duplication is inevitable. There are 379+ known red cells antigens with a number of weakened forms of these antigens described in literature. Detail explanation may be obtained from current texts in blood group serology.

Exercises (222):

Match each of the blood group systems or rare antigens in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. Blood system includes variant antigens such as Cl(KL), Matthews (Js^b), and Ul^a.
___ 2. This antibody is almost always of the incomplete variety of antibody and is usually most reactive in the indirect Coombs.
___ 3. This antigen is present in approximately 9 percent of the population, and has been an occasional cause of hemolytic transfusion reaction and HDN.
___ 4. Accounts for the two blood types of the Duffy system.
___ 5. This antibody can sometimes only be recognized with the use of antihuman serum, whereas albumin or trypsin methods are inadequate.
___ 6. The two antigenic factors comprising this system are designated Fy^a and Fy^b.
___ 7. Antibodies of this system have caused delayed transfusion reactions when they have not been present in demonstrable levels at the time of transfusion.
___ 8. The blood bank technician is generally concerned with the negative and positive forms of this factor in the Kidd system.
___ 9. The factors of this system is not common in transfusion reactions.
___ 10. This antibody has been characterized as an IgA antibody which accounts for its failure to cross the placenta.
___ 11. This antigen deteriorates more rapidly than any other antigen on red cells.
___ 12. Anti-I antibodies may obscure the pattern of a given antigen of this system.
___ 13. Cold temperatures enhance the reaction of this antigen and the strength of this antigen varies considerably on the different positive persons.
___ 14. These antigens occur infrequently and are referred to as family antigens such as Levay, Gr, Jobbins, Mi^a, Becker, Di^a, and Chr^a.
___ 15. These antigens are known as high incidence antigens. Examples of such antigens are: Vel(Ve^a), I-i, Auberger(Au^a), and Js^b.

Column B

a. Jk^b factor.
b. Public antigens.
c. Lutheran system.
d. Kidd system.
e. Kell system.
f. P system.
g. Duffy system.
h. K factor.
i. Fy^a factor.
j. Anti-Fy^a.
k. Private antigens.
l. P_1.
m. Anti-Lu^b.
n. Anti-K.
o. Jk^a.

CHAPTER 3

Transfusion and Transfusion Practices

THE TRANSFUSION of blood did not become an accepted and effective medical practice until about 50 years ago. Yet, throughout history attempts were made to transfuse blood. One of the best known attempts in earlier times took place in 1490. A physician tried to rejuvenate Pope Innocent VIII by getting him to drink the blood of three youths. According to historical accounts, the Pope died, the donors died, and the attending physician fled the country.

Today blood transfusion therapy in clinical practice has changed considerably. Whole blood is composed of several cellular and soluble elements each with its own specific function. The development of methods for preserving red blood cells and other blood components has made transfusion readily available.

The main purpose of the blood bank technician is to prepare blood and blood components for transfusion. In this chapter, we will investigate the use of checks and balances to safeguard the preparation of these components. This safeguard is needed foremost to protect the patient who receives blood and blood components and ultimately the technician who prepares them.

3-1. Specific Blood Banking Procedures

After what we have said in Chapters 1 and 2, it would be an understatement to say that blood is a complicated, immunologic substance. The practical blood banker makes use of the accumulated knowledge of antigens and antibodies to provide valuable diagnostic data to the physician. He also makes blood a safe product for transfusion. In this section, we will elaborate on particular laboratory tests closely associated with blood banking.

223. Point out the physical factors which prevent the binding process of antigens and antibodies, and the principle of the Coombs test; briefly state some of the processes in the production of Coombs serum.

Coombs Test. An observable reaction cannot occur between an antigen and its corresponding antibody unless both are suspended in a suitable medium. Certain physical factors prevent the binding process. Theoretically, two of the most significant factors that keep antigens and antibodies apart are (1) repelling electrical forces among the colloidal particles and (2) the size of the molecules, or specifically the dimensions of the antibodies considered.

Principle of the test. Coombs, Mourant, and Race introduced antihuman globulin serum (AHG) to detect red cells sensitized by incomplete antibodies. If red cells are incubated with an incomplete antibody directed against one of the red cell's antigens, the antibodies attach themselves to the red cells, sensitizing but not agglutinating them. The antihuman globulin serum (AHG), as you know, is called Coombs serum. The way in which AHG acts is illustrated in foldout 1 printed and bound in the back of this volume, which describes the direct (detail A) and indirect (detail B) Coombs test. The only significant difference between the direct and indirect Coombs test is that, in the direct Coombs test, we are concerned with cells which have been coated with antibody *in vivo*. In the indirect Coombs test, we are testing serum for Coombs-reactive antibodies or *in vitro* sensitization of red cells. In the past, the term "immune incomplete antibody" was often used in describing AHG-reactive antibodies. However, there are other antibodies that fail to react in either saline or anti-γ-globulin serum, and they are also called by the imprecise term "incomplete antibodies." There are also IgM antibodies (for example, Lewis) that must be detected with anti-IgM globulin serum or by complement-binding techniques. Thus, you can easily see that what is detected with Coombs serum depends upon the specificity of the Coombs serum you are using. Most available antihuman serum contains both anti-γ-globulin fractions. A broad spectrum Coombs serum should also contain anti-complement (C') complement-binding antibodies.

You may consider that the antigen-antibody reaction taking place on the red cell remains invisible. To confirm the fact that antibody-antigen reaction has actually taken place on the red cell surface, Coombs serum is added. The originally invisible antigen-antibody reaction is thus made visible.

Manufacture. Coombs serum can be prepared in a number of different animals. The most popular are rabbits, goats, and sheep, selected on the basis of handling ease, good immunization response, and the lower costs involved with these domestic animals. The principles of AHG production are essentially those with which you are familiar. The antigen is human globulin, which is foreign to the animal. The antigen is fractioned from human plasma. The human gamma globulin stimulates the animal to produce an antibody, antihuman globulin. After the antibody has been produced in a rabbit, the collection of rabbit serum can be continued for years if periodic stimulating doses of antigen are given. Since each rabbit's response to the antigen is different, it is necessary to blend and standardize the resulting antihuman globulin reagent. To be of commercial worth, the antibody must be of high titer so that it can be diluted and still sold as an effective reagent.

Exercises (223):

1. What are the two given significant factors that keep antigens and antibodies apart?

2. Briefly describe the principle of the Coombs test.

3. Briefly explain the difference between the direct and indirect Coombs.

4. Name the three animals most often used in the production of Coombs serum.

5. What antigen is used to stimulate animals in the production of Coombs serum?

6. Why is it necessary to blend serum from several different animals in producing Coombs serum (for example, several rabbits)?

224. Indicate whether given statements correctly reflect the guidelines for performing the Coombs test and possible sources of error.

Coombs Testing. When you perform a Coombs test, it is very important that you wash the test cells completely free of globulin. At least three washings are necessary to accomplish this. Further, you should *never* use your finger or your thumb to stopper the tube when you are mixing the cell suspension. By so doing you may introduce extraneous human globulin. This could result in a negative Coombs test because the globulins from body secretions neutralize the antihuman globulin of the Coombs serum. Use a rubber stopper or paraffin film when mixing cell suspensions. Commercially prepared control cells or saline-washed, anti-D coated, group O (Rh positive) cells should be used. Preservation is possible with modified Alsever's reagent. Commercial preparations are by far more reliable. Always run controls with every test. Do not overcentrifuge the cells. Store Coombs serum at refrigerator temperature because it deteriorates rapidly at room temperature. Follow the instuctions that accompany the AHG reagent, especially with regard to storage and incubation time.

Sources of Error. In addition to some of the points already mentioned, there are a number of sources of error which produce false-positive and false-negative Coombs tests. Some reasons for a false-positive reaction are:

a. Bacterial contamination of any component, for example, cells, serum.

b. Scratches in the glassware of traces of silica, which may produce the agglutination phenomenon.

c. Use of saline with a concentration in excess of 0.85 to 0.9 percent.

d. The presence of multivalent cations such as ions of copper, zinc, and iron.

Some causes of a false-negative Coombs test are:

a. Inadequate washing of the red cells.
b. Contamination of the antihuman globulin.
c. Incubation temperature above or below the optimum for the antibody being detected.
d. Inadequate incubation.
e. Absence of active serum complement.
f. Elution of the antibody of prolonged incubation.

Exercises (224):

Indicate whether each of the following statements is true (T) or false (F). If your answer is false, explain your answer.

T F 1. It is very important that the test cells be washed completely free of globulin.

T F 2. Mixing the cell suspension with the use of your finger or thumb will enhance the quality of reaction with the Coombs test.

T F 3. Since the Coombs test has high specificity, no controls are necessary.

T F 4. Overcentrifugation will not adversely affect the Coombs test, and is recommended.

T F 5. The presence of multivalent cations such as ions of copper, zinc, and iron may produce a false positive Coombs.

T F 6. Scratches in the glassware of traces of silica may produce a false-negative Coombs.

T F 7. Incubation temperature above or below the optimum for the antibody being detected may cause a false-positive Coombs.

T F 8. Elution of the antibody by prolonged incubation may cause a false negative Coombs.

225. Identify the drugs associated with the development of a positive direct Coombs test in terms of the given characteristics and properties.

Drug-Induced Positive Coombs Test. Individuals taking certain drugs may have a positive direct antiglobulin test, with or without red cell destruction, with or without circulating antibody activity. The AABB Seminar on Problems Encountered in Pretransfusion Tests provides a detailed review of mechanisms, methods, and references; this section is a brief summary of notable findings.

Alpha-methyldopa and mefenamic acid. Alpha-methyldopa (the antihypertensive drug Aldomet) and mefenamic acid (the analgesic drug Ponstel) may induce formation of an antibody which is serologically indistinguishable from the IgG auto-antibody of warm autoimmune hemolytic anemia and which may persist for 6 months or longer after cessation of the drug. Approximately 15 percent of patients taking α-methyldopa develop a positive direct antiglobulin test after 3 to 6 months of drug exposure, but of these, less than 1 percent have evidence of hemolysis. The antibody, which reacts with the patient's cells and nearly all other normal human red cells, appears to have some Rh-directed specificity.

Immune complex adsorption. Immune complex adsorption onto red cells may occur with a heterogeneous group of drugs including phenacetin, quinidine, PAS, sulfonamides, isonicotinic acid, and chlorpropamide. The drug induces formation of a specific antibody, usually IgM and capable of complement binding. The antibody, once formed, combines with circulating drug, and the drug-antibody, complex attaches to red cells. The attachment is rather loose, so the complex may elute off the cells, but the complement remains fixed to the cells and may cause intravascular hemolysis or may produce a positive antiglobulin test. Very small amounts of drug may initiate this process once the antibody has been formed, but these difficulties persist only while the drug is present.

Drug antibodies combine with red-cell bound drugs to produce agglutination or a positive antiglobulin test. Drugs such as penicillin, cephalothin, and others induce formation of specific IgG antibodies, usually not complement binding. These drugs, in large doses, bind firmly to red cell surfaces. The antibody combines with the drug wherever they interact. If the drug is adsorbed to red cells, the antibody appears to be reacting with the cell.

Drugs causing protein adsorption. Drugs (notably cephalothin) may cause nonimmunologic adsorption of protein. Through an unexplained mechanism, this drug and its related compounds may cause the indiscriminate adsorption of proteins to the cell surface. The phenomenon is related to dose and duration of therapy, and as many as 3 to 5 percent of patients receiving cephalothin may develop a positive antiglobulin test in this fashion. This persists as long as large amounts of the drug are present, and disappears gradually after cessation of therapy. Red cell life span is not shortened.

Exercises (225):

Match each of the drugs in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

_____ 1. Approximately 15 percent of patients taking this drug develop a positive direct Coombs after 3 to 6 months of therapy.

_____ 2. An analgesic drug which may induce an antibody serologically identical to the IgG autoantibody of warm autoimmune hemolytic anemia.

_____ 3. A hypersensitive drug which may induce an antibody serologically identical to the IgG auto-antibody of warm autoimmune hemolytic anemia.

_____ 4. Group which induces formation of a specific antibody, usually IgM and capable of complement binding.

_____ 5. Process in which an antibody formed by a drug combines with the circulating drug; and the antibody complex then attaches to the red cells.

_____ 6. These drugs induce formation of specific IgG antibodies, usually not complement binding.

_____ 7. May cause nonimmunologic adsorption of protein to the cell surface.

Column B

a. Penicillin.
b. Cephalothin.
c. Phenacetin, quinidine, PAS, sulfonamides, isonicotinic acid, and chlorpropamide.
d. Alpha-Methyldopa (Aldomet).
e. Mefenamic acid (Ponstel).
f. Immune complex adsorption.

226. Indicate whether given statements correctly reflect the principle and method of antibody detection.

Antibody Studies. The detection, titration, and identification of immune antibodies in the sera of recipients and blood donors are of prime importance in the procurement of compatible blood for patients requiring a transfusion. Immune blood groups and antibodies are principally of the IgM and IgG classes, and they are initiated by the infusion of red cells, pregnancy, parental injection of whole blood and immune gamma globulin. Let's discuss the detection of antibodies.

Antibody Detection. The qualitative presence of antibodies in the serum is detected by the use of appropriate reference cells. These cells can be pooled locally or procured commerically. The use of commercial preparations is recommended because some antigens are relatively rare and will not be included unless the cell pool is taken from a fairly large number of donors. Cell pools stored at 4° C must be prepared fresh every 2 weeks. There are several reasons for detecting antibodies: the presence of a discrepancy between cell and serum ABO grouping; crossmatch studies; preparation of low-titer, O-negative blood; prenatal workups and jaundice in a newborn; and any other routine situation in which it is desirable to screen for antibodies. Irregular blood group antibodies result from previous transfusions, parental injections of blood, and the isoimmunization of pregnancy.

Reference cells for antibody studies should be representative of the important antigens. In the Rh system, antigens D, C, c, E, and e must be included. One commericially available cell reagent provides two vials of erythrocytes, one of the type R_1 (DCe) and the other type R_2 (DcE). It really doesn't matter what combination of cells is present as long as all of the antigens are there. However, this is not the only reason you should use commercial reagents rather than pooled O cells. Other antigens should also be present in the reagent cells. These include M, N, S, s, U, P, K, k, Kp^b, Js^b, Fy^b, Jk^a, J^b, Le^a, Le^b, Lu^b, Yt^a, Vel(Ve^a), I, Ge, and Xg^a. At least one commercial preparation that contains all of these antigens is available. Since some antigens are relatively rare, you can easily see the difficulty one might have in putting together his own reagent cells. Further, it is inadvisable from a legal standpoint to prepare your own blood bank reagents, unless licensed by the FDA to manufacture such products.

Procedure. In a conventional procedure for antibody detection, place 2 drops of the serum to be tested in a small tube. (If your reagent test cells are separated into two vials, for example, phenotype R_1, and R_2, you must set up two tubes.) Add 1 drop of reagent test cells to each of the tubes. Centrifuge the tube without incubation and read. Some procedures call for incubation at 37° C for 15 minutes at this point, while other methods follow immediately with the protein phase. Add 2 drops of 22 percent bovine albumin to one of the two tubes, unless you prefer to set up the albumin and Coombs in separate tubes apart from the saline phase. After centrifugation, look for agglutination or hemolysis, and then wash the cells three times with saline. Be careful to decant the saline completely after the last wash. You can do this by standing the tube upside down on a paper towel. After washing, add 2 drops of Coombs serum to each tube, centrifuge, and reexamine for agglutination or hemolysis, indicative of a positive reaction. It is a good idea to read the Coombs test microscopically as well as macroscopically. When you read a cell suspension microscopically, the use of a cover slip helps distribute the cells and permits clear observation of individual cells. A stereoscopic microscope is helpful when you are reading cell suspensions in a tube. This not only permits easy viewing but also allows comparison of one tube with another and eliminates the necessity to transfer the mixture to a slide. If the Coombs test is negative, add 1 drop of a 5 percent suspension of AHG-positive control cells to validate the Coombs system.

Some methods for detecting antigen-antibody reactions call for incubation at 4° C at one point in the procedure. This is done to detect antigens that react only at low temperature, for example, MNS. You must distinguish agglutination at 4° C from nonspecific cold agglutinins by warming the agglutinated cells to 37° C. If the agglutination dissipates, it is not due to a *specific* cold-reacting antibody. Controls of the patient's cells and serum should be included. With each antibody screen and crossmatch, an additional control should be used. This control tests the patient for the agglutination of his own cells, and consists of the patient's own cells and serum. This patient control insures that the reaction obtained in the screen is not due to something in the patient's serum.

Exercises (226):

Indicate whether each of the following statements is true (T) or false (F). If you indicate false, explain your answer.

T F 1. Immune antibodies detected are principally of the IgA and IgE classes.

T F 2. These immune antibodies are initiated by infusion of red cells, pregnancy, parental injection of whole blood, and immune gamma globulin.

T F 3. Reference cells for antibody studies should be representative of all available antigens.

T F 4. It is inadvisable from a legal standpoint to prepare your own blood bank reagents unless licensed.

T F 5. It is a good idea to read the Coombs microscopically only.

T F 6. The stereoscopic microscope permits easy viewing and allows comparison of one tube with another.

T F 7. If the Coombs test is negative, the tubes are then discarded and the test reported.

T F 8. You may distinguish agglutination at 4° C from nonspecific cold agglutinins by warming the agglutinated cells to 37° C.

T F 9. If agglutination dissipates upon warming the agglutinated red cells, it is due to a specific cold-reacting antibody.

T F 10. It is not necessary to include controls of the patient's cells and serum.

T F 11. An additional control should be used with each antibody screen and crossmatch.

T F 12. The patient control insures that the reaction obtained in the screen is due to something in the patient's serum.

227. Describe antibody identification in terms of the given principles, procedures, and interpretation of results.

Antibody Identification. Antibodies are identified by tests of the serum in which they occur with a panel of cells containing known antigens. The cell panels are obtained from biological suppliers, usually on a contract basis so that a fresh supply is always available. Each vial in the panel of cells contains erythrocytes with certain specified antigens. A series of tubes is set up in approximately the same manner as for the antibody screening test. A complicating feature of antibody studies is that each vial of cells contains more than one specified antigen. To identify a particular antibody, the technician must interpret his results by the process of elimination. In general, reactions in albumin are usually caused by antibodies of the Rh system.

Method. Add serum to a panel of cells suspended in saline solution and incubate at 22° C and 37° C. Note the cell panel used in table 3-1. If cold antibodies are suspected, place the mixture of serum and cells in a refrigerator at 4° C, but use tubes having only antigens that are likely to react in the cold, as for example, Lewis, MN or P. Add serum to panel cells suspended in albumin (22 percent); incubate at 37° C and follow with antihuman globulin test (AHG). Add serum to enzyme treated cells and follow in AHG technique. For an autologous cell control, the patient's own red cells must be tested in parallel with the panel of cells by each technique simultaneously.

Examine macroscopically and microscopically for agglutination or hemolysis. The degree of agglutination should be noted. Further, examine for effect of dosage. Varying degrees of reaction intensity also suggest multiple antibodies or dosage. By *dosage* we mean that a stronger reaction is observed with cells from a homozygous individual (for example, cc or MM) than from a heterozygous person (Cc or MN). Some antibodies are more common than others, react best in certain media and at certain temperature. Some idea of the type of antibody may be obtained from table 3-2, frequency table of antibody reactions.

Conclusions. If the control cells are negative and the panel cells are positive, one or more antibodies are present. The conditions indicated below may be applicable:

a. Some cells positive, some negative; control cells negative: One or several antibodies are present. With single antibodies, the pattern of reaction is usually evident; however, the possibility of additional antibody(ies) should not be ignored.

b. All panel cells are equally positive; patient control negative: An antibody against a high frequency antigen may be present. Reference laboratories may be helpful.

c. All panel cells positive at room temperature, much weaker at 37° C; antiglobulin phase variable; autocontrol negative or weakly positive: A cold antibody may be present.

d. All panel cells and patient control (autocontrol) positive at room temperature, weaker at 37° C; antiglobulin phase variable: A cold antibody with autologous activity may be present.

e. All panel cells and autocontrol are positive in antiglobulin phase: The patient may have a warm autoimmune antibody.

f. Cells on panel are negative or variably positive; autocontrol positive in antiglobulin phase: The

TABLE 3-1
SIMPLIFIED CELL PANEL (a single antibody)

Patient's group: O **Type: rh (cde)**

	Antigenic Determinants									Results			
Panel cell	Rh (D)	rh′ (C)	rh″ (E)	(c)	(e)	K	k	Fy^a	Fy^b	Saline 37 C	HP	AGT	En-zyme
1	+	O	O	+	+	O	+	O	O	O	+	3+	3+
2	O	O	O	+	+	+	+	O	+	O	O	O	O
3	O	+	O	+	+	O	+	+	O	O	O	O	O
4	+	+	O	O	+	O	+	+	+	O	O	3+	3+
5	+	O	+	+	O	O	+	+	O	+w	2+	4+	4+
6	O	O	+	+	+	O	+	O	+	O	O	O	O
Autologous red cells										O	O	O	O

O = No agglutination HP = High protein AGT = Antiglobulin test

patient's cell may have a positive antiglobulin test at the same time that an alloimmune antibody is present in the serum.

Exercises (227):

In questions 1 through 3, complete each sentence with the appropriate word or words. A phrase rather than a single word may be required for the correct response.

1. Each vial in the panel of cells contains erythrocytes with certain specified ______________ .

2. In the cell panel, a series of tubes is set up in approximately the same manner for the ____________ ____________ test.

3. To identify a particular antibody, the technician must interpret his results by __________________ .

4. If cold antibodies are suspected, tubes containing what antibodies should be used?

5. What should the autologous cell control consist of?

6. What is dosage?

7. What type of antibody is present in the panel cells if all are equally positive and the patient control is negative?

8. What type of antibody may be present if all panel cells are positive at room temperature, much weaker at 37° C; antiglobulin phase variable and the autocontrol is negative or weakly positive?

9. What type of antibody may be present if all panel cells and autocontrol are positive in antiglobulin phase?

228. Specify the significance of varying strengths of antibody reactions using panel cells, and identify the antibody present from given results obtained on a cell panel.

Procedure for Identification. Test results obtained with a cell panel should be accurately graded and recorded after each phase of testing. Different strengths of reaction, or reactions in different phases of testing, may indicate multiple antibodies, or they may be due to a single antibody showing the property of dosage. Let us use table 3-1 in this procedure to identify the antibody shown in the panel.

TABLE 3-2
FREQUENCY TABLE OF ANTIBODY REACTIONS

Technic	Temp. C	% Frequency of positive reactions with random blood samples				
		0-10	10-50	50-75	75-90	90-100
Saline and/or albumin	4-22	Lu^a i	Le^a	Le^b	P_1 MN	I
Saline and/or albumin	22-37	$rh^{w\prime}(C^w)$ Lu^a	rh''(E) Le^a S	rh'(C) Le^b	$Rh_o(D)$ hr'(c) s	hr''(e) Lu^b Vel
Saline and/or albumin plus antiglobulin	37	$rh^{w\prime}(C^w)$ K Kp^a Lu^a Di^a Y^b	rh''(E) Le^a S Js^a	rh'(C) Le^b Fy^a Jk^b Xg^a Do^a	Rh (D) hr'(c) Fy^b s Jk^a Au	hr''(e) k U Kp^b Ge^a Lu^b Yt^a Vel Js^b Di^b
Enzyme	37	$rh^{w\prime}(C^w)$	rh''(E) Le^a	rh'(C) Le^b	$Rh_o(D)$ hr'(c)	hr''(e)
Enzyme plus antiglobulin	37	$rh^{w\prime}(C^w)$	rh''(E) Le^a	rh'(C) Le^b Jk^b Do	$Rh_o(D)$ hr'(c) Jk^a	hr''(e)
Hemolysis saline-suspended cells	37		Le^a	Le^b Jk^b	Jk^a	Vel
Hemolysis enzyme-treated cells	37		Le^a	Le^b Jk^b	Jk^a P_1	
All technics						
All technics and temperatures			Le^a	Le^b		P, P_1P^k

Interpretation. Follow these steps for antibody identification:

1. Cross out all antigenic determinations present in the first red cell sample that did not react with the serum, in this example No. 2.

D C E ~~c~~ ~~c~~ ~~K~~ ~~k~~ Fy^a ~~Fy^b~~

2. Repeat with the next negative sample, in this example No. 3, thus eliminating rh' (C) and Fy^a.

D ~~C~~ E ~~Fy^a~~

3. Repeat with the next negative sample, in this example No. 6, thus eliminating rh" (E).

D ~~E~~

4. Consider only the antigenic determinant that has not been crossed out.

D ~~C~~ ~~E~~ ~~c~~ ~~c~~ ~~K~~ ~~k~~ ~~Fy^a~~ ~~Fy^b~~

Do all red cells reacting possess this determinant? Yes.
Do all red cells possessing it react? Yes.
Does the patient lack it? Yes.

5. Antibody identification: Anti-Rh_o(D).

Exercises (228):

1. What do different strengths of reaction or reactions in different phase of testing in the cell panel indicate?

2. What would the antibody be recorded and identified as, using table 3-1 if the cell reaction results were as follows?

	Results			
	Saline 37° C	*HP*	*Agt*	*Enzyme*
1.	0	0	0	0
2.	0	0	0	0
3.	0	+	3+	3+
4.	+w	+	3+	3+
5.	0	0	0	0
6.	0	0	0	0

229. Cite the purpose and frequency for use of enzymes in antibody studies.

Use of Enzymes. Some antibodies are best detected by the use of enzymes. Treatment of red cells with enzymes may cause them to agglutinate by incomplete blood group antibodies. The effect of enzymes is, presumably, to remove ionogenic surface groups and thus reduce surface charge. Their use depends upon the situation and judgment of the technician. Enzymes are not recommended in routine compatibility testing or as a replacement for the antiglobulin test. Enzymes can also confuse the crossmatch picture, since in many instances they enhance the reaction of nonspecific agglutinins and also prevent the reaction of antibodies in the MNS and Duffy systems by destroying their receptor sites. Enzyme preparations are available commercially. However, the pH is critical, and if not optimal, antibodies may not be detected. You should use enzymes only when testing for suspected antibodies that are known to be enzyme reactive.

Exercises (229):

1. What specific purpose does the use of enzyme serve in the treatment of red cells?

2. When should you use enzymes in antibody screening procedures?

3. Why is the use of enzymes not recommended in routine compatibility testing or as a replacement for the Coombs test?

230. Point out the meaning of absorption and elution in terms of their significance, purposes, and techniques in antibody studies.

Absorption. Absorption is removing antibody from a serum by reacting it with the specific antigen, and then physically separating the antigen-antibody complexes from the serum. Intact red cells are the usual source of antigen, but red cell stroma is occasionally used. Situations in which absorption may be useful include the following:

a. Removal of cold or warm autoantibody activity, to permit evaluation of coexisting allo-antibody.

b. Removal of anti-A and/or anti-B from a serum which contains an unexpected antibody suitable for reagent use.

c. Separation of mixed antibodies in serum or eluate.

d. Documentation that cells contain an antigen through demonstrating their ability to remove antibody from serum.

Serologic considerations. The temperature should be that at which the strongest reactions occur. If both cold and warm antibodies are present, separation is enhanced by absorbing the cold activity at 4° C or the warm activity at 37° C. The usual volume is one part undiluted serum to one volume of washed packed cells. For complete absorption of high-titered antibodies, diluted serum may be more efficient. With weak antibodies, the volume of undiluted serum should be greater than the volume of red cells.

Technique. Wash selected red cells at least three times with isotonic saline. If the red cells were in contact with serum or plasma containing cold antibodies, washing at 37° C may be indicated. Incubate the packed red cells and serum at the optimum temperature for the reaction, agitating frequently. Centrifuge the specimen mixture. If absorption is at 4° C, use prechilled centrifuge cups or a refrigerated centrifuge. Centrifugation at high temperatures may result in elution of antibody from the red cells. After centrifugation, the serum is immediately removed completely. Once the antibody has been absorbed, the serum should be tested for complete absorption against a freshly prepared suspension of red cells used for absorption. If an eluate is to be made, save the adsorbing cells.

Elution is the removal of an antibody that has been adsorped onto red cells either *in vivo* or *in vitro*. Elution is useful to demonstrate and identify antibodies on the red cells of umbilical cord blood or infant's blood in hemolytic disease of the newborn. Also, elution may be used to demonstrate and identify antibodies in a mixture. In all elution methods, the most critical detail is the complete removal of unadsorbed antibodies surrounding the red cells. Most eluates are prepared at 56° C; however, when the cells are to be used for further testing, the elution should be carried out at 42° to 44° C, since the antigen sites may be destroyed at 56° C. The red cells should be washed at the same temperature at which the elution is performed.

Exercises (230):

1. What is absorption?

2. List some of the situations in which absorption may be useful.

3. What is the usual volume in ratio for performing the absorption procedure?

4. In performing the absorption technique, if the red cells were in contact with serum or plasma containing cold antibodies, at what temperature should washing be done?

5. If absorption is to be done at 4° C, what will result if centrifugation is done at higher temperature?

6. What is elution?

7. What are some useful purposes of elution?

8. At what temperature are most eluates prepared, and at what temperature should the red cells be washed?

231. Indicate whether given statements correctly reflect the principle and factors affecting antibody titrations.

Antibody Titration. Titration is a semiquantitative means of measuring the amount of antibody. Serial dilutions (usually twofold) of antibody are tested with a constant volume of red cells, and the result is expressed as the reciprocal of the highest dilution at which macroscopic agglutination is observed. It is worthwhile to point out that the volume of cell suspension is not considered in calculating titers in blood banking. This is in contrast to serological titers, in which the cell suspension is taken into account in calculating the dilution factor. Table 3-3 illustrates a serial dilution for anti-A and anti-B. The last tube or highest serum dilution showing definite agglutination is reported as the titer of the antibody tested. If no agglutination is present in the 1:16 dilution, the titer should not be recorded. In this case, lower dilutions, for example, 1:2, 1:4, and 1:8, should be prepared to determine the exact end point.

Titration is most often used (1) to demonstrate changing amounts of antibody during pregnancy; (2) to clarify differences between alloactive and autoactive antibodies in a serum as, for example, in identifying anti-I activity; (3) to identify *least incompatible* donor units when crossmatch difficulties exist.

Factors Affecting Titrations. Titration of antibody is dependent upon certain factors. The technician should keep in mind the following considerations:

a. Ideally, cells from the same donor, freshly drawn and prepared, should be used for each titration. If commercially prepared reagent cells are used, the same genotype should be employed consistently, and the cells should be used within the first few days of their shelf life.

b. If the antibody is diluted with saline, the red cells should be suspended in saline. If a high-protein medium is used for dilution, the red cells should be suspended in albumin or serum.

c. Meticulous pipetting technique is necessary for meaningful titration results. Pipettes should be of the *to contain* type (the semiautomatic pipettes are recommended) so that the material in each tube can be thoroughly mixed by aspiration, which also serves to rinse out all active material in the pipette. A clean pipette or pipette tip should be used for each tube.

d. Results should be read macroscopically. The prozone phenomenon may produce weaker reactions in the first one or two tubes than in higher dilutions, so the entire series of tubes should be evaluated, starting with the most dilute and ending with the most concentrated sample.

e. Optimum incubation time, temperature and centrifugation condition should be determined in preliminary evaluation of the antibody. Once determined, these should be used consistently.

f. If serums are to be compared, the titrations should be done at the same time. With prenatal specimens, successive samples should be stored frozen for comparison with subsequent specimens. Each specimen should be tested along with the immediately preceding sample. A titer change of less than two tubes is not significant.

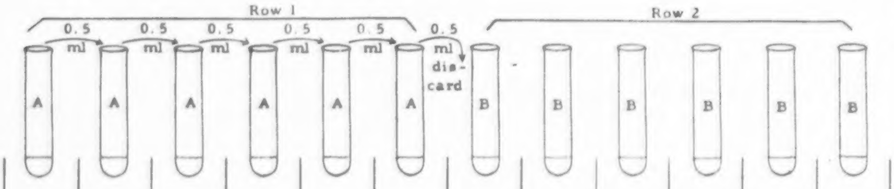
Exercises (231):

Indicate whether each of the following sentences is true (T) or false (F). If you indicate false, explain your answer.

T F 1. Serial dilutions of antibody are tested with a constant volume of red cells.

T F 2. The result of the titration is expressed as the reciprocal of the lowest dilution at which macroscopic agglutination is observed.

TABLE 3-3
ANTI-A AND ANTI-B SERIAL DILUTION



Clean Test Tubes	A	A	A	A	A	A	B	B	B	B	B	B
Normal Saline, ml		0.5	0.5	0.5	0.5	0.5						
Serum Being Tested	1:16 Dil. 0.5 ml	1:16 Dil. 0.5 ml	0.5 ml of: 1:32	1:64	1:128	1:256						
Serum Dilutions	1:16	1:32	1:64	1:128	1:256	1:512						
Diluted Serum Transferred From Row A to Row B (ml)	0.25 →	0.25 →	0.25 →	0.25 →	0.25 →	0.25 →						
Group A Cells 2 Percent Suspension	0.25	0.25	0.25	0.25	0.25	0.25						
Group B Cells 2 Percent Suspension							0.25	0.25	0.25	0.25	0.25	0.25

Centrifuge all tubes at 2000 rpm for 2 minutes. Gently dislodge the cells and observe macroscopically for clumping.

T F 3. As in serological titers, the volume of cell suspension is considered in calculating titers in blood banking.

T F 4. The last tube or serum dilution showing definite agglutination is reported as the titer.

T F 5. Titration may be used to identify *least incompatible* donor units when crossmatch problems exist.

T F 6. Ideally, test cells used in titration should preferably be prepared from reagent cells with the same genotype.

T F 7. If the antibody is diluted with saline, a high protein medium should be used for the cell suspension.

T F 8. Results should be read microscopically, if necessary.

T F 9. If serums are to be compared, the titration should be done at different times for validity.

T F 10. Once the incubation time, temperature, and centrifugation condition has been determined in preliminary evaluation, variations in subsequent determination are permissible.

3-2. Processing Blood for Transfusion

The blood transfusion service of any hospital is one of the most important areas of activity in routine, as well as in emergency, situations. As a medical laboratory technician, you will be called upon to perform quickly and accurately in blood banking. We will describe the basic requirement of securing blood in the next chapter.

There are many clinical requirements for blood transfusions. While you should be generally familiar with the reasons for administering blood (shock, blood loss, anemia, etc.), the need is determined by a physician. Your job is to provide blood in the quantities required and perform the necessary laboratory tests to meet medical and legal specifications. At times this can be very demanding, especially when a large number of units are needed within a limited time. A properly performed crossmatch procedure can take 1 hour or more, and this is not always realized by personnel outside the blood bank laboratory. Every blood bank needs an effective system of standard operating procedures, and the system must be followed to the letter. In other words, the blood bank laboratory should be a scientific and orderly operation, not a series of crises. As in any well-organized laboratory section, there is a valid requirement for records and paperwork.

232. Specify the advantages of maintaining blood bank records and cite the importance and use of SF 518 and the blood bank ledger.

Transfusion Service Records. Each blood transfusion service must develop a system of record keeping that best serves its needs. Records must provide accurate, complete, step-by-step accounts of work relating to blood and blood products, donor operations, and patient care. Keeping accurate and complete records is of legal importance as well as of administrative value. Documented incidents and experience have shown that most errors in blood banking are either clerical errors or misidentification of patient or donor. Records are of value in the following ways:

a. They permit orderly and effective administration of the blood transfusion service.

b. Records explain actions involving donors and patients; for example, they provide a history of blood bank procedures pertaining to the donor, the patient, and to the units of blood.

c. Accurate records reduce medical errors which could be harmful to the donor or patient.

Two of the most important records in the blood bank are DD Form 572, Blood Donor Record, and SF 518, Medical Record—Blood or Blood Component Transfusion. We will discuss DD Form 572 in Chapter 4 because the record card is particularly applicable to the blood donor. However, as we shall see this is by no means the only value of DD Form 572.

SF 518, Blood or Blood Component Transfusion. The SF 518 has three sections. Section I, Transfusion Requisitions, includes brief remarks on patient history when applicable and the signature of the person who verifies that the blood specimen for the crossmatch is obtained from the person whose name appears on the tube label. Section II, Blood Type, Compatibility Information and Certification, is the laboratory portion. Section III, Record of Transfusion, includes such information as the signature of the person starting the transfusion, the amount of component given, and the time transfusion is completed. A sample copy of SF 518 is printed in foldout 2 printed and bound at the back of this volume.

Section I should always be completed and the patient's identification written or printed on the SF 518 *before* a pilot tube of blood is drawn from the patient. Fewer errors are made if Section I is completed in full and kept with the patient's pilot tube during processing. All pilot tubes must bear a permanent label. It is a dangerous practice to work with unlabeled tubes. If someone brings you an unlabeled tube of blood and says, "This is from Mrs. Jones," you had better be very certain that it is indeed from the Mrs. Jones you think it is from. We do not mean to imply that in emergency situations the completion of a form has priority over all else, but there are very few emergencies that preclude the proper use of the SF 518. The few minutes needed to complete it are not usually that critical. In some cases, the physician himself prepares the requisition on SF 518, but Section I of this form is often prepared by other hospital personnel charged with the responsibility of carrying out the physician's orders. As always in your work, be aware of administrative errors. An assistant may, for example, specify *Whole Blood* when the physician really wants packed cells or red blood cells. Though you cannot be a mind-reader, experience may lead you to tactfully clarify any request that seems to be in error. A thoughtful technician respects paperwork, but he does not consider it an end in itself. The patient's welfare is his prime consideration.

Section II of SF 518 is completed as you perform compatibility tests. In some hospitals, it is the practice to inform the physician when blood is ready. Ordinarily, this depends upon whether the request is routine or *Stat.* The first thing you must determine when you receive a requisition is how quickly the blood is needed. What is done to complete the crossmatch and what must be done after it is completed are determined by the time element. The person who accepts a request has the responsibility for appropriate action. A request for blood is never taken lightly or set aside in deference to a coffee break; *Followup* action is mandatory.

The blood transfusion form is printed in sets of three, with carbon paper between the copies. The original or first copy is labeled, "Medical Record Copy"; the second copy is labeled, "Return to Transfusion Service With Blood Product Container"; and the third copy is labeled, "Transfusion Service Copy." When you send a unit of blood to the ward, to surgery, or wherever it is needed, you keep the copy labeled "Transfusion Service Copy" in the laboratory as a suspense copy, and the other two copies are forwarded with the unit. When the blood is given, the physician signs the SF 518, and the copy labeled "Return to Transfusion Service With Blood Product Container" is returned with the used blood bag or component container. At this time, you file the completed form and destroy your suspense copy. Each day you check your suspense copies. If a record has not been cleared (if the completed second copy has not been returned), you may have to trace the unit of blood. Blood that is not administered is sometimes kept in refrigerators in surgery or on the ward. This is not permitted unless such refrigerators meet all the requirements prescribed by the American Association of Blood Banks for the storage of blood. We will discuss most of these requirements in Chapter 4. Moreover, some blood bank directors prefer as a matter of safety to keep crossmatched blood under blood bank control and to release units only as they are needed for transfusion.

Blood bank ledger. Another transfusion service record, in addition to the two forms already mentioned, is a blood bank ledger. The purpose of the blood bank ledger, as a compatibility record, is to indicate that the number of units of blood or blood products requested have been successfully crossmatched and are available. There is no standard form for this purpose. However, as a compatibility record of laboratory testing, it should include:

a. The patient's name, hospital number, age, sex, physician.

b. Recipient's ABO group and Rh type.

c. Donor's number, ABO group, Rh type, and units crossmatched.

d. Crossmatch information including test performed, results and interpretation, and the date of the test.

e. Signature or initials of person doing the tests. Columns are headed with all pertinent information, as illustrated in figure 3-1. A record book of this type consolidates all of the operational information necessary to control the issuing of blood. Take a few minutes to study figure 3-1.

Exercises (232):

1. List the advantages of keeping good blood bank records.

2. Name three vital record documents used in the blood bank.

3. What section of the SF 518 shows the signature of the person who verifies that the blood specimen is obtained from the patient for whom the crossmatch is intended?

4. What section of the SF 518 shows the signature of the person who verifies the crossmatch?

5. If you are the blood bank technician and someone brings you two unlabeled tubes of blood and says, "This is for Mrs. Brown," whose name appears on the SF 518, what immediate action should you take?

6. What section of SF 518 is completed as you perform the compatibility tests?

7. What must be first determined when you receive a SF 518 request for blood?

8. When you send a unit of blood to the ward or to surgery, how many copies are sent with the unit, and how many copies are returned to the laboratory after the blood is given?

9. What is the overall purpose of the blood bank ledger?

233. Indicate whether the given statements correctly reflect the guidelines for maintenance and retention of transfusion service records.

Maintenance. AFM 160-24, *Standards for Blood Banks and Transfusion Services,* establishes and maintains uniform blood banking standards for the military departments that will be compatible with those of the civilian field of blood banking. Uniformity has become essential with the advent of the Food and Drug Administration's requirement for licensing of blood banks and issuance of the National Blood Policy by the Department of Health, Education, and Welfare. Thus, recommendations for maintenance and retention of transfusion service records are issued in accordance with the directives of AFM 160-24.

Each blood bank and transfusion service should develop a system of record keeping which best serves its needs, in terms of the scope and size of the workload involved. The record system should make it possible to trace a unit of any blood or blood component from donor to recipient, and to recheck the laboratory records applying to the specific product.

Retention. Records to be retained by the blood bank must include SFs 518 and DD Forms 572. Transfusion records should be kept on all patients. The SF 518 is a true record of transfusion and should meet the requirement of a transfusion record. Accreditation requirements and the standards of the American

RECIPIENT														DONOR			CROSS MATCH		ISSUE						DISPOSITION		
					REACTIONS							RESULTS															
TRANS-FUSION NR	DATE	NAME	WARD	SER-VICE	ANTI A	ANTI B	A CELLS	B CELLS	ANTI RHO (D)	ALB CON	ABO Gp	RHO (D) TYPE	ANTIBODY SCREEN	BLOOD GROUP	RHO TYPE	UNIT NUMBER	TIME & DATE COMPLETED	TECH	TIME	DATE	INDIVIDUAL ACCEPTING CUSTODY	REC'D SERVICE	TECH ISSUING BLOOD	TECH	RET'D	CANCEL	USED

BLOOD BANK LEDGER

Figure 3-1. Sample blood bank ledger.

Association of Blood Banks provide for a transfusion committee. Hence, some system must permit the orderly review of transfusion records on all patients. The following guidelines pertain to SFs 518, DD Forms 572 and to other essential records for quality control and administration of the blood transfusion service:

a. Records of each potential recipient's ABO and Rh type must be retained permanently and be available for immediate reference minimally for the duration of the current hospitalization.

b. Records of potential recipients with significant unexpected antibodies must be maintained in the blood bank, and be available for immediate reference, for at least 5 years.

c. Information concerning the following phases of the transfusion service shall be recorded and appropriately retained for at least 5 years. Legal requirements for retention of records vary in different states.

(1) Donor history, examination, release, and reactions (DD Form 572).

(2) Transfusion request (SF 518).

(3) Transfusion compatibility and release (issue) (SF 518).

(4) Administration of blood or blood components (SF 518).

(5) Adverse reactions to transfusions (SF 572).

(6) Refrigeration temperature.

(7) Quality assurance records.

(8) Blood inspection.

(9) Blood and components received from outside sources (other than the inpatient facility).

(10) Disposition of unused blood.

(11) Laboratory tests. The actual *results* observed with each test as well as the final *interpretation* shall be recorded (SF 518).

Exercises (233):

Indicate whether each of the following statements is true (T) or false (F). If you indicate false, explain your answer.

T F 1. AFM 160-34 establishes standards for blood banks and transfusion services.

T F 2. With the advent of the Food and Drug Administration's requirement for licensing of blood banks and issuance of the National Blood Policy by HEW, diversity among blood banks has become essential.

T F 3. The record system should make is possible to trace a unit of any blood or blood component from donor to recipient.

T F 4. Transfusion records should be retained only on patients with irregular antibodies.

T F 5. Records of potential recipient with significant unexpected antibodies must be maintained in the blook bank, be available for immediate reference, and retained for at least 2 years.

T F 6. Information concerning donor history transfusion request and adverse transfusion reactions shall be recorded and retained for at least 5 years.

234. Identify the given blood components in terms of their clinical uses, storage temperature, and expiration time.

Blood Components. Blood component therapy has been a significant advancement in improved patient care. Blood component therapy refers to separating the elements of the blood and transfusing these elements of the blood to patients as needed. For example, many patients need only the red blood cells; in such instances, the physician may specify transfusion with packed erythrocytes. The plasma can then be processed and fractionated for other purposes. Refer to table 3-4, and note the many other items that can be obtained from a unit of whole blood. Because blood is such a precious commodity, it should not be wasted. The economy in the use of blood components can more than offset the cost of preparation. Components that can be made available in the blood bank include packed red blood cells, frozen red blood cells, leukocyte poor red blood cells, single donor plasma, single donor fresh frozen plasma, single donor cryoprecipitate, whole blood (cryoprecipitate and/or platelets removed), single donor plasma (cryoprecipitate removed), platelet rich plasma, and platelet concentrate. Let's discuss these components.

Red blood cells. Red blood cells (human) is the official name of this product. This product is that which remains after removal of the plasma from whole blood. The plasma may be separated following centrifugation or undisturbed sedimentation at any time before the expiration date of the blood.

Packed red blood cells are the product of choice for any patient with severe anemia, a patient who does not require restoration of blood volume. Thus, packed red blood cells are the product of choice for most patients with chronic anemia, congestive heart failure, and elderly or debilitated patients in whom rapid shifts of blood volume are not well tolerated.

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TABLE 3-4
CHART OF BLOOD COMPONENTS

Component	Clinical Uses	Storage Temperature	Unit Contents/ Active Substance per Transfused Unit	Risk of Hepatitis	Expiration Time
Red cells (Packed Red Blood cells)	Increase patient red cell mass. Exchange transfusion. Anemia without hypovolemia or with hypervolemia. Any bleeding with circulatory overload potentials. Problems of electrolyte balance who still need blood replacement.	1° to 6°C	250 to 350 ml/200 ml packed Red cell mass)	Same as whole blood	21 days in closed system or 24 hours in an open system.
Leukocyte-poor blood	Prevent febrile reactions from leukoagglutinins.	1° to 6°C	200 to 250 ml/185 ml red cells	Same as whole blood	21 days closed. 24 hours open.
Frozen Red blood cells	Increase red cell mass, prevent tissue antigen sensitization, prevent febrile or anaphylactic IgA reaction, provide rare bloods.	-80° or -130°C	200 ml	Less than whole blood	3 years frozen. 24 hours thawed.
Single Donor plasma	Blood volume expansion	1° to 6°C	220 to 250 ml	Same as whole blood	5 years.
Pooled plasma	None--No longer available	--	--	Greater than whole blood	--
Single Donor fresh frozen plasma	Treatment of coagulation disorders	-30°C	220 to 250 ml/ Factor VIII (220 to 250 units)	Same as whole blood	2 hrs thawed. 12 months frozen.
Single donor cryoprecipitate	Hemophilia & von Willebrand's disease, fibrinogen deficiency	-18°C	10 to 25 ml/80 to 100 units Factor VII	Same as whole blood	4 to 6 hours thawed. 12 months frozen.
Platelet concentrates	Bleeding due to thrombocytopenia	Room temp (30 ml) 1° to 6°C (30 ml)	30 to 50 ml/5.5 X 10^{10} platelets or more	Same as whole blood	6 to 72 hours depending on storage.
Albumin	Blood volume expansion-replacement of protein	1° to 6°C	250 or 50 ml/12.5 gm albumin	Less than whole blood	3 to 5 years.
Fibrinogen	Hypofibrinogenemia	1° to 6°C	Stated on vial	Greater than whole blood	5 years.
Immune Serum Globulin	Passive protection by providing high titer antibodies. Agammaglobulinemia	1° to 6°C	Varies with patient's weight	Less than whole blood	3 years.
Anti-Hemophilic Factor (AHF) Concentrate	Hemophilia	--	Lyophilized/125 to 750 units of AHF	Greater than whole blood	Dated period.
Whole blood	Severe anemia with hypovolemia. Coagulation factor deficiency (Factors VII, IX, X, or XI)	1° to 6°C	500 ml	--	21 days (ACD or CPD) solutions. 48 hours (heparinized).

Packed red cells may be prepared from blood collected in ACD (acid-citrate-dextrose) or CPD (citrate-phosphate-dextrose) anticoagulant solutions. Single unit plastic bags may be used, but multiple-unit bags are better. A primary bag accompanied by several satellite bags is especially useful for pediatric transfusions.

The packed cells must be stored at an average ambient temperature of 1° to 6° C. If the seal is broken, the cells must be used within 24 hours after removal of plasma. Otherwise, the same 21-day expiration date as whole blood is used.

Leukocyte poor red blood cells. Patients who receive multiple transfusions, or multiparous women may develop antibodies to antigens contained on leukocytes and platelets. When patients with leukocyte antibodies receive blood containing incompatible leukocytes, a febrile transfusion reaction can occur. These leukocyte reactions do not cause red blood cell hemolysis, but can be extremely uncomfortable for the patient. Leukocyte-poor red blood cells are indicated for patients with febrile transfusion reactions due to leukocyte antibodies.

Leukocyte-poor blood is the component remaining after removal of most of the leukocytes and platelets from whole blood. Several methods are available to separate the leukocytes and platelets from whole blood. With most techniques, plasma is also removed along with some of the red blood cells. Frozen red blood cells are convenient since 83 to 93 percent of the leukocytes are removed by processing these cells. Differential sedimentation or centrifugation may be used to remove the leukocytes.

Method of storage and expiration date are the same as red blood cells.

Remember that the two most common uses for leukocyte-poor blood are: (a) to prevent transfusion associated febrile reactions where anti-leukocyte antibodies have been demonstrated or are suspected; and (b) to prevent sensitization to tissue antigens in potential transplant recipients.

Single donor plasma. Single donor plasma is separated from an individual collection of whole blood. No attempt is made to maintain the activity of the labile clotting factors. The plasma is removed from blood no later than 5 days after the expiration date of the whole blood. Single donor plasma from which cryoprecipitate has been removed is an equivalent component. The plasma may be frozen and stored at -18° C or lower for no more than 5 years.

Single donor plasma has been used for expansion of blood volume and is indicated in the treatment of shock due to loss of plasma in burns, peritoneal injuries, acute pancreatitis, mesenteric thrombosis, and in the initial treatment of shock due to hemorrhage while the blood is being crossmatched.

Single donor fresh frozen plasma. Single donor fresh plasma is separated from whole blood within 4 hours of collection. This component if not used immediately should be frozen and stored at -18° C or colder. Both fresh, and fresh frozen plasma contain all of the plasma clotting factors. Labile clotting factors such as factors V and VII will disappear upon storage in the non-frozen state. Although transfusion is given without a crossmatch, it must be compatible in terms of the recipient's ABO group. The plasma should be transfused within 2 hours of thawing. These products are indicated in the treatment of clotting factor deficiencies V, XI, and XIII, and in the treatment of other clotting deficiencies, such as factors VII, VIII, IX, and X when other specific concentrates are not available. Fresh frozen plasma may be stored for 1 year after collection date, in contrast to single donor plasma which may be stored at -18° C for 5 years.

Single donor cryoprecipitate. Cryoprecipitate is prepared by thawing a unit of fresh frozen plasma at 4° C and then recovering the cold-precipitate factor VIII protein by centrifugation. After the separation of the plasma from the cold-insoluble materials under sterile conditions, the container is sealed and refrozen within 4 hours. When this product is maintained at -18° C or below, it shall be stored no longer than 12 months from the time of donation of the original unit of blood.

Cryoprecipitate is useful in the treatment of patients with classic hemophilia, von Willebrand's disease, Factor XIII deficiency, and decreased fibrinogen levels. The cryoprecipitate should be infused as soon as possible after it has been thawed in order to avoid loss of activity.

Whole blood (cryoprecipitate and/or platelets removed). Although separated components are preferred for transfusion therapy, plasma may be processed for cryoprecipitate and/or platelets and the supernatant plasma may be returned to the red blood cell component in a closed system using a technique that will insure sterility. Under such conditions, the label must indicate that cryoprecipitate and/or platelets have been removed. The standards for whole blood shall apply.

Single donor plasma (cryoprecipitate removed). Single donor plasma provides a closed system or other technique that insures sterility is used. Plasma that has been frozen, thawed, and separated from its cryoprecipitate may be used for transfusion purposes. The label shall indicate that cryoprecipitate has been removed. The standards for single donor plasma shall apply.

Platelet rich plasma. This is plasma prepared by centrifugation at a force and for a time known to leave most of the platelets in the supernatant plasma. Patients with thrombocytopenia, due to inadequate platelet production, who are bleeding frequently will benefit from platelet transfusion. Prophylactic transfusion may be of value in patients undergoing intensive cancer chemotherapy. Either daily or every other day platelet transfusions may be necessary.

Platelets possess three varieties of antigens. Some antigens are unique to platelets. Further, platelets share ABO antigens with red cells and other tissues and HL-A antigens which are present on all tissues except erythrocytes.

If available, it is preferable to give platelets from ABO compatible donors. However, satisfactory clinical results have also been observed with ABO incompatible platelets and one should not hesitate to use them if necessary.

There is little evidence that platelets possess the Rh$_o$(D) antigen, and theoretically this antigen may be ignored in platelet transfusion.

Platelet concentrate. This preparation contains at least 50 percent of the total number of platelets in the original unit of fresh whole blood suspended in less than 50 ml of plasma.

If stored at room temperature, continuous gentle agitation of the platelet concentrate shall be maintained throughout the storage period.

Frozen red blood cells. Red blood cells to which an appropriate amount of a cryoprotective agent, such as glycerol solution has been added, may be stored at ultra-low temperatures for long periods of time. Prior to transfusion, they are thawed, and the glycerol is removed by a washing technique. Freezing arrests the metabolism of the cells, while glycerol-deglycerolization removes the plasma and the great majority of the nonerythrocytic formed elements.

The freezing process allows blood to be frozen and stored for autologous transfusion, or for stockpiling of rare red cell types.

Exercises (234):

Match each blood component in column B with the statements in column A by placing the letter of the column B item beside the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. This component is considered the product of choice for any patient with severe anemia who does not require restoration of blood volume.

___ 2. If the seal is broken, for the removal of plasma, this product must be used within 24 hours.

___ 3. This type product is indicated for patients with febrile transfusion reactions due to leukocyte antibodies.

___ 4. Differential sedimentation or centrifugation may be used to remove the leukocytes in this preparation.

___ 5. Plasma is removed with no attempt to maintain the activity of the labile clotting factors.

___ 6. May be stored for 5 years at -18° C or lower.

___ 7. Used as a blood volume expander in the treatment of shock due to loss of plasma in burns, and peritoneal injuries.

___ 8. May be used in the initial treatment of shock due to hemorrhage while the blood is being crossmatched.

___ 9. Is indicated in the treatment of clotting factor deficiencies such as factors V, XI, and XIII.

___ 10. May be stored for 1 year after collection at -18° C or lower.

___ 11. Is prepared by thawing a unit of fresh frozen plasma at 4° C and then recovering the cold-precipitated factor VIII protein by centrifugation.

___ 12. Is useful in the treatment of patients with classic hemophilia, von Willebrand's disease, factor XIII deficiency, and decrease fibrinogen levels.

___ 13. Plasma may be processed for cryoprecipitate and/or platelets, and the supernatant plasma may be returned to the red blood cell component in a closed system.

___ 14. A closed system or sterile technique whereby frozen plasma has been thawed and separated from its cryoprecipitate.

___ 15. After centrifugation at a force and a specified time most of the platelets remain in the supernatant plasma.

___ 16. If stored at room temperature, continuous agitation of this component shall be maintained throughout the storage period.

___ 17. Components to which an appropriate amount of a cryoprotective agent such as glycerol solution has been added and stored at ultra-low temperatures for a long period of time.

___ 18. This component may be stored for autologous transfusion, or for stockpiling of rare red cell types.

Column B

a. Leukocyte poor red blood cells.
b. Single donor plasma.
c. Whole blood (cryoprecipitate and/or platelets removed).
d. Single donor plasma (cryoprecipitate removed).
e. Platelet rich plasma.
f. Single donor fresh frozen.
g. Red blood cells.
h. Single donor cryoprecipitate.
i. Frozen red blood cells.
j. Platelet concentrate.

235. Cite the twofold purpose of the compatibility test, the components and their significance, and list some errors that the crossmatch will detect.

The Crossmatch. Sometimes referred to as a compatibility test, the crossmatch is a test between the blood of a person who is to receive a transfusion and the blood of a donor. The compatibility test, consisting of a series of procedures performed by the Blood Bank before transfusion to insure proper selection of blood for the patient, has a twofold purpose: the prevention of a transfusion reaction and the assurance of maximum benefit of the transfusion to the patient. The test is performed to reveal a possible incompatibility between the donor's blood and the recipient's blood. Agglutination or hemolysis on the major side (donor's cells and patient's serum) or on the minor side (patient's cells and donor's serum) is considered evidence of incompatibility. If the blood is incompatible, it is not administered to the patient. In place of the minor crossmatch, a broad spectrum, donor antibody screening test is often performed. The minor crossmatch may achieve a status of near extinction if the principle of adequate donor screening is strictly followed. The antibody screening done by the collecting facility will detect most antibodies the donor may have and the unit will subsequently be removed from stock or be transfused only as packed cells. In any case, the donor's serum must be adequately tested for immune antibodies. By *adequately*, we mean that the test should be carried out in saline, albumin, and AHG at room temperature, at 37° C, and at lower temperatures for both specific and nonspecific cold agglutinins.

Procedures vary somewhat as methods improve. Therefore, no one method can be learned as the ultimate in technique. But it is of the greatest importance that you intelligently apply the prescribed and accepted method of the facility in which you are working. Never attempt to modify a procedure or take shortcuts that are not authorized. To do so would be to assume unnecessary responsibility and perhaps even jeopardize the life of the patient.

Review of Basic Technique. A complete crossmatch includes saline, high-protein, and antihuman globulin

TABLE 3-5
ANTIBODY DETECTION IN VARIOUS PHASES OF A CROSSMATCH PROCEDURE

CROSSMATCH PHASE	PRINCIPAL ANTIBODIES DETECTED
SALINE MEDIUM	
Room Temp.	ABO incompatibilities Cold agglutinins, P, MNS Lewis, Lu, Wright.
At 37°C	Most Rh-Hr, Lewis Differentiates cold agglutination.
HIGH PROTEIN	Rh-Hr
ANTI-HUMAN GLOBULIN (AHG)	Most Rh-Hr Duffy, Kidd, Kell, and certain other antibodies are detected only by AHG.

test systems. Enzymes may also be included, but should never be considered a substitute for one of the other phases of a crossmatch. Those techniques considered optimal for detection of hemolyzing and agglutinating antibodies include saline, or serum suspensions of donor cells incubated with recipient serum at room temperature (18° - 25° C) for 15 to 30 minutes. The optimal method for detecting coated antibodies is the use of antiglobulin serum following incubation at 37° C for 15 to 30 minutes. This incubation may be carried out in a potentiating medium of high dielectric constant, such as 22 to 30 percent albumin, and customarily read before washing and addition of anti-human globulin serum. Antigen-antibody reactions that take place in these media are thus detected, as illustrated in table 3-5. You should understand what the crossmatch will detect and what it will not detect. Review these points:

a. The crossmatch will detect:

(1) Antibodies present in the recipient serum directed against antigens on the donor red cells.

(2) Some errors in ABO grouping, labeling, and identification of donors and recipients.

b. The crossmatch will not:

(1) Insure normal survival of donor red cells.

(2) Prove that recipient or donor serum is free of irregular antibodies.

(3) Prevent the recipient immunization.

(4) Detect all ABO grouping errors.

(5) Detect errors in the Rh typing of either recipient or donor unless the serum contains an Rh antibody.

(6) Detect all errors in identification (administrative errors). Moreover, a compatible crossmatch is no assurance that isoimmunization will not occur.

Exercises (235):

1. What is the twofold purpose of the compatibility test?

2. What media are commonly used in the crossmatch procedure?

3. The major side of the crossmatch consists of what components?

4. What test is often performed in place of a minor crossmatch?

5. What optimal method in the crossmatch is used for detecting coated antibodies?

6. Before washing and addition of antihuman serum, in what medium is the incubation carried out providing a high dielectric constant?

7. What are some factors that the crossmatch will detect?

8. What assurance does a compatible crossmatch give that isoimmunization will not occur?

236. Indicate whether given statements correctly reflect your responsibilities as a blood bank technician in terms of monitoring the condition of the blood, patient identification, and the availability of crossmatched blood.

Responsibilities of the Crossmatch Technician. Human errors are more common than technical ones in a Blood Bank or Transfusion Service. Nontechnical mistakes, such as inadequate or incorrect identification of the recipient or donor, can usually be traced to a break in rules or to inadequacy of regulations.

Your job as a blood bank technician is to provide the physician with a blood product that will be beneficial and safe for a patient. The test tubes represent the patient's circulatory system. If an incompatibility is detected in your test tube crossmatch, the dire consequences of a transfusion reaction taking place in the patient are predictable. Extreme caution and demanding concentration are essential requirements in the blood bank. Before you provide a unit of blood for use, you must check it for contamination and hemolysis visually. Inspect plastic bags for leaks or defects. Check all identifying numbers and log the blood out properly. Further patient identification should be established before you issue a unit of blood for transfusion. This may be done by issuing blood to legally responsible hospital staff members only and requiring that they submit a request slip containing the intended recipient's name, date, ward, and register number.

AFM 168-4, *Administration of Medical Activities*. requires that blood earmarked for a patient will be held in a laboratory for 48 hours after being typed and crossmatched. It is then made available for retyping and recrossmatching for another patient. Of course, in certain specified cases the physician may require that blood be held for a longer period. When blood is released, the physician should be advised of this action because—strictly speaking—only the physician who orders blood has the authority to release it. Never overcommit a particular unit of blood. To crossmatch the same unit of blood for more than one patient simultaneously is to ask for trouble.

Exercises (236):

Indicate whether each of the following sentences is true (T) or false (F). If you indicate false, explain your answer.

T F 1. Technical errors are more common in Blood Bank or Transfusion Service than human errors.

T F 2. If an incompatibility is detected in your test tube crossmatch, the dire consequences of a transfusion reaction taking place in the patient are predictable.

T F 3. Extreme caution and demanding concentration are essential requirements in the blood bank.

T F 4. Visually checking the blood for contamination and hemolysis is not necessary since this cannot occur after the blood is drawn and refrigerated.

T F 5. Patient identification should be established before you issue a unit of blood for transfusion.

T F 6. Crossmatched blood will be held for no more than 24 hours before being released for another patient.

T F 7. It is advisable in the interest of economy to crossmatch the same unit for more than one patient.

237. Indicate whether given statements correctly reflect the guidelines for the emergency crossmatch and the emergency release of blood.

The Emergency Crossmatch. If a surgical patient hemorrhages or suffers any other sudden blood loss, the blood bank is called upon to furnish replacement blood as soon as possible. The physician may demand uncrossmatched blood on the basis of his clinical judgment and his evaluation of the urgency of the situation. It is part of the physician's job to accept full responsibility for this action.

AFM 168-4 states, "It is imperative that final typing and crossmatching be accomplished prior to all transfusions. The typing and crossmatching should be doublechecked and countersigned by a person familiar and current with the detailed techniques and procedures being utilized."

Emergency release of blood. When time does not allow crossmatching, ask the physician to sign a release form acknowledging his responsibility and accepting the risks in giving uncrossmatched blood. This action helps protect you legally from personal liability in this situation. Obtain a sample of blood from the patient and from the pilot tube or blood bag segment before the unit leaves the bank and begin a routine crossmatch. At the first sign of incompatibility, notify the physician. A form designated "Emergency Blood/Component Request" may be devised from the information given in AFM 160-50, *Technical Methods and Procedures of the American Association of Blood Banks.* Such a release does not absolve the blood bank from its responsibility to issue properly grouped or labeled blood. If necessary, issue the uncrossmatched blood. Notify the blood bank physician or chief of the blood bank that uncrossmatched blood has been released. Thus, in an extreme emergency when there is not time to determine the patient's blood group, group O Rh-negative blood may be given if it has had at least 70 percent of the plasma removed or is free of hemolytic anti-A and anti-B. O Rh-positive blood may be issued only if O Rh-negative blood is not available. In addition, group specific blood should be given if time permits testing the patient in the transfusion facility without reliance on previous records. Blood group cards from other facilities or groups from *dog tags* or driver's licenses *must not* be accepted as evidence of blood group.

Begin the routine compatibility testing procedure. If the clinician cannot wait the length of time required for a complete crossmatch, release the blood at whatever stage of testing it is in, continuing the crossmatch after release. If the blood specimen from the patient has not clotted sufficiently, one drop of liquid thrombin per milliliter of serum or the amount of dry thrombin adhering to an applicator stick may be added to cause rapid clot formation.

Emergency crossmatch guide. Emergency crossmatching techniques can be used to prepare blood when time is short. The following guide is recommended in handling emergency situations; however, local policy should be followed:

If a 60 minute period is available, the blood is released following the full saline, albumin, Coombs crossmatch.

If a 30-45 minute period is available, the blood is crossmatched but the procedure is shortened by reducing the incubation times. The blood may be released after a 10 minute incubation, followed by the antiglobulin phase.

If a 15-30 minute period is available, the patient's ABO group and Rh type should be performed and uncrossmatched type-specific blood released after the saline and albumin phase. Continue toward completion.

If a 5 minute period or less is available, give ABO group and Rh type-specific blood uncrossmatched. Begin complete crossmatch. If necessary, give group O Rh-negative blood with at least 70 percent of the plasma removed or free of hemolytic anti-A and anti-B.

If blood is released without the complete crossmatch, appropriate records must reflect this action. When a patient is transfused, there is some risk, either immediately as a transfusion reaction or as subsequent sensitization. The omission of crossmatch safeguards increases that risk. Deciding when to transfuse blood is entirely the responsibility of the physician, and the blood banker.

Exercises (237):

Indicate whether each of the following sentences is true (T) or false (F). If you indicate false, explain your answer.

T F 1. The typing and crossmatching should be double checked by any person not necessarily familiar with the procedures being utilized.

T F 2. A sample of blood is obtained from the patient and from the blood bag segment before the unit leaves the bank.

T F 3. When the physician signs a release for uncrossmatched blood, this absolves the blood bank from its responsibility to issue properly grouped or labeled blood due to the rush.

T F 4. When there is not time to determine the patient's blood group, group O Rh-negative blood may be given if it has at least 30 percent of the plasma removed.

T F 5. When group specific is given, blood group cards from other facilities or groups from *dog tags* or driver's licenses must be accepted as evidence of blood group.

T F 6. If a 30-45 minute period is available for the emergency crossmatch, the blood may be released after a 10 minute incubation followed by the antiglobulin phase.

238. Cite some basic causes of an incompatible crossmatch, the procedures for preliminary investigation of incompatible crossmatch, and some technical causes of incompatibility.

Incompatible Crossmatch. When incompatibility is seen in an early phase of a crossmatch, the crossmatch may be completed, to give information as to temperatures and media where reactions occur, variability of reactions, and the percentage of incompatible donors. These clues will aid in choosing correct conditions for antibody identification work.

It is preferable to determine the cause of the incompatibility rather than crossmatch blindly. If the clinician informs the laboratory personnel that the blood need is too urgent for this course of action, many random units may be crossmatched. If possible, attempts to identify the antibody should be started while the crossmatch is being completed.

Preliminary investigation. Recheck ABO groups and Rh types of incompatible donors and recipients; recheck prior sample numbers against donor units. Run an autocontrol (patient control) with the antibody identification panel, with the next series of crossmatches.

An incompatible crossmatch is detected by agglutination or lysis of the donor or recipient's red cells at any phase of the crossmatch.

Causes of incompatibility. Some of the causes of an incompatible crossmatch and suggested followup action are given in table 3-6. Some incompatible crossmatching may be due to an error in ABO grouping of donor or recipient, error in identification of the donor's or recipient's specimen, irregular antibodies, and technical errors. A suggested work outline for incompatible crossmatch has been presented in AFM 160-50.

Irregular antibodies. As you may recall, these irregular, unexpected, or atypical antibodies are not normally present in the serum of a particular blood group; for example, anti-A_1 in the A_2B group or anti-Rh_o in a Rh-negative individual. These atypical antibodies may react with the donor's red cells in the major crossmatch, while they react also in the donor's serum with the recipient's red cells in the minor crossmatch. As previously indicated, the donor's or the recipient's red cells may not contain the specific antigens that match the irregular or atypical antibodies in the serum of either recipient or donor. Thus, a compatible crossmatch does not rule out the presence of atypical antibodies.

Some Technical Causes. False positive reactions may be caused by the following: dirty glassware, bacterial contamination, chemical or other contaminants in reagents (including saline), fibrin clots, and overcentrifugation.

TABLE 3-6
CROSSMATCH INCOMPATIBILITY PROBLEMS

Observed Incompatibility	Some Possible Causes	Action Required
I. Saline or serum room temperature	(1) ABO error	(1) Front type and Back type donor and recipient
	(2) Cold agglutinin	(2) Allow recipient blood to clot in refrigerator (b) Agglutination dissipates at 30-37 degrees C.
	(3) Irregular antibody	(3) Identify antibodies with reagent cells (e.g. "Panocell")
II. Saline, serum or high protein at room temperature.	(1) Irregular antibody	(1) Same as I (3)
	(2) Autoagglutinin	(2) Auto-adsorption; see I (2)
	(3) Rouleaux	(3) Add saline
III. AHG or Enzyme	(1) Irregular antibody	(1) Same as I (3)
	(2) Autoagglutinin	(2) Auto-adsorption; see I (2)
	(3) Positive direct Coombs	(3) Check direct Coombs on donor or recipient as indicated
IV. Any phase	Technical errors	Investigate following: Dirty glassware; bacterial contamination; chemical contamination; fibrin clots; over-centrifugation or centrifuge vibration.

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Exercises (238):

1. Why should the crossmatch be completed when incompatibility is seen in an early phase?

2. What checks are made in the preliminary investigation of incompatibility?

3. List some causes of an incompatible crossmatch.

4. Why is the presence of atypical antibodies not ruled out with a compatible crossmatch?

5. What are some causes of false positive reactions?

3-3. Adverse Effects of Blood Transfusion

A transfusion reaction is defined as any unfavorable event occurring in a patient during or following transfusion of blood or blood products. Since compatibility testing is performed for the detection of antibodies to red cell antigens, the adverse effects of transfusion are most commonly caused by the other elements of blood, such as leukocytes, platelets, plasma proteins, and any infectious agents that may be present in the donor blood. In addition, every transfusion carries a risk of isoimmunization to the elements of blood, and transmission of disease.

239. Identify the given causes of immediate and delayed transfusion reactions and transmission of disease in terms of their characteristics and features.

Transfusion Complications. Transfusion complications fall into two major groups: (1) immediate transfusion reaction, seen within 48 hours of blood administration; and (2) delayed transfusion reactions and transmission of disease. When a transfusion reaction is suspected, the transfusion should be discontinued and the remaining blood, together with the reaction report, should be sent to the blood bank for laboratory investigation. If a severe reaction occurs, the clinical staff should call the blood bank immediately.

Immediate Hemolytic Transfusion Reaction. The classical signs and symptoms of a hemolytic transfusion reaction are indicated when: the patient flushes, complains of pain in the extremity receiving the blood, pain in the back, constricting pain in the chest; profound clinical shock may or may not be present.

Immune hemolysis is usually the result of a reaction between an antibody in the recipient's plasma and the red cells of the donor; occasionally, it may be the antibody in the donor's plasma that reacts with the recipient's red cells. The red cell destruction may be intravascular, where the red cells are hemolyzed directly in the blood stream, by the action of antibody and complement, leading to the release of hemoglobin and other portions of the red cell into the plasma (for example, ABO); or extravascular, when the red cells, after reaction with antibody, are removed by the reticuloendothelial system. This leads to hyperbilirubinemia but little or no release of hemoglobin into the plasma (for example, $\mathrm{Rh_o}$, Kell). Hemolysis may initiate disseminated intravascular clotting, leading to the consumption of coagulation factors and fibrinolysis. Massive transfusions may also lead to bleeding by depletion of platelets and dilution of coagulation factors. Since the early signs of a hemolytic reaction may be the same as those of febrile nonhemolytic reactions, such as chills and fever, blood infusion should be stopped if these signs appear and an investigation begun. Investigation procedure will be discussed later in this section.

Hemolytic events due to nonimmunologic causes are not uncommon. Nonimmune hemolysis may be due to overheating, infused blood exposure to extreme osmotic stress, or to trauma. It rarely produces the life-threatening complications of hemolysis initiated by antigen-antibody reactions. Still, contaminated plastic sets or intravenous fluids can occur, and this possibility should be considered when other investigations are unproductive.

Anaphylactic and Allergic Reactions. When transfusion of blood or plasma is given, the recipient receives more than forty different plasma proteins, and alloimmunization to certain plasma proteins may occur. The cause of *simple* allergic reactions, usually manifest only by urticaria (hives), is far from clear. This is an immediate effect. Immune complexes from reactive antibody in either the recipient or the donor probably stimulate tissue mast cells to release histamine, which produces the vasodilatation responsible for the skin weals. Allergic reactions considered an immediate transfusion reaction are treated with antihistamines, and may be minimized by using packed red cells, minimizing the dose of infused antigen.

Bacterial Reactions. Bacteria may cause transfusion reactions in two ways: by producing febrile reactions due to pyrogens and toxins which are breakdown products of bacteria; or by causing serious, often fatal reaction, when the donor blood is contaminated with large numbers of living organisms. Even when the best possible precautions are taken during collections, it has been reported that approximately 2 percent of units are contaminated at the time of collection. Fortunately, most of these organisms do not survive the bactericidal action of the fresh donor blood storage at 4° C. Septic reactions are often caused by psychrophilic organisms, that is to say, those capable of growing at low temperatures, for example, pseudomonads, coliforms, and achromobacters. They are usually endotoxin-producing gram-negative bacilli that can metabolize citrate as a sole source of carbon.

Contamination of the blood with bacteria may be grossly obvious, as the supernatant plasma may be dark brown or red and the cells may be obviously discolored. However, the infected blood may look normal both macroscopically and microscopically. Platelets stored at room temperature have been reported to cause bacterial sepsis following transfusion. When bacterial contamination is suspected, microscopic examination and culture of donor blood is indicated. When infected blood is transfused, symptoms appear quickly (10-30 minutes): chills, headache, vomiting, muscle pains, diarrhea, and high fever. Prompt, energetic treatment is required.

Room temperature storage of platelet concentrates may increase the risk of significant bacterial growth. When fever and chills complicate transfusion of platelets which have been stored at room temperature, contamination must be considered and investigated.

Circulatory Overload. Even though we, as technicians, are not directly involved with administering the unit, we must be aware of problems concerned with transfusion of compatible units that cause circulatory overload. Sudden increases in circulating blood volume are not well tolerated by certain patients, particularly patients with cardiac or pulmonary diseases, very anemic patients, or infants. Whole blood transfusions in these patients may cause dyspnea, coughing, pulmonary edema, and cyanosis. If these symptoms develop, the transfusion should be stopped, and treatment for circulation overload instituted. If the transfusions have to be continued, patients with severe anemia should be transfused with concentrated red cells at a rate no faster than 70-100 ml/hr.

Delayed Hemolytic Reactions. A previously immunized recipient may have little or no circulating antibody at the time compatibility tests are performed. When blood is transfused, no reaction occurs, but the transfusion may provoke an anamnestic immune response so that after several days there is a rapid increase in antibody and thus increased destruction of the transfused red cells. These reactions most often occur 5 to 7 days after the transfusion, occasionally as late as 10 to 11 days, and have caused acute renal failure. When large amounts of blood have been transfused, the majority of the red cells in the patient's blood are involved in a subsequent delayed hemolytic reaction, and the picture may closely resemble autoimmune hemolytic anemia.

Viral hepatitis. Viral hepatitis, transmitted from donor blood to a recipient, constitutes at the present time the most common and serious problem of transfusion practice. Components such as platelets, AHF cryoprecipitate, AHF concentrates, plasma, and fibrinogen may be responsible for hepatitis transmission, with the risk proportioned to the number of donors involved. Serum albumin, plasma protein fraction, and globulin are regarded as safe derivatives, since the method of their preparation inactivates hepatitis virus.

The incidence of hepatitis virus carrier state in the blood donors is generally higher in commercial blood donors than in voluntary blood donors. High morbidity and mortality result from post-transfusion *serum* hepatitis caused by hepatitis B virus with an incubation period of 50 to 180 days. Transmission of hepatitis A virus causes *infectious hepatitis* within a short incubation period of 15 to 50 days. Frequently, it is difficult to distinguish between these two types of viral hepatitis. The discovery of Australia antigen and its association with post-transfusion *serum* hepatitis has provided a laboratory means of detecting the infectious blood. Methods for laboratory detection of Australia antigen, referred to as hepatitis B antigen (HB_sAg), will be discussed in a subsequent section.

Cytomegalovirus (CMV) infection. Following the transfusion of large amounts of fresh blood, particularly in cardiovascular surgery, the patients may develop a mild illness associated with fever, splenomegaly, neutropenia, and the presence in the blood of atypical cells resembling those found in infectious mononucleosis. This syndrome is sometimes referred to a postperfusion or postcardiotomy syndrome. About 6 to 12 percent of donors have CMV infected leukocytes.

Syphilis. Transfusion syphilis does not appear to be a serious risk in transfusion therapy today. Refrigeration has a spirocheticidal effect, and, therefore, the greatest risk of syphilis comes from the use of freshly collected blood units and platelet preparations. The conventional cardiolipin tests for reagin yield no information regarding infectivity. In fact, spirochetemia is more likely to be found before the serologic tests become positive.

Malaria. Malaria can be transmitted by the transfusion of whole blood, packed red cells, platelets, concentrates, or other blood components which contain red blood cells. All four species of the malarial parasite have been implicated. Since no practical laboratory tests are presently available to exclude infective donors, a careful history must be relied upon.

Miscellaneous Acute Problems. Many acute problems have been associated with massive blood transfusion including "citrate intoxication," hyperkalemia, hypothermia, and pulmonary insufficiency due to debris in stored blood. In addition, air embolism, rare in this era of plastic transfusion apparatus, may still occur. Hemolysis due to overheated blood has been reported, as have rare episodes of hemolysis due to infusions of abnormal red cells (for example, G6PD deficient). Red cells from patients with hemoglobinopathies and membrane defects may survive poorly, but apparently do not hemolyze immediately.

Exercises (239):

Match each of the types of reaction and following causes of transfusion reaction in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

____ 1. Reactions seen within 48 hours of blood administration.

____ 2. In this general transfusion reaction, the red cell destruction may be intravascular where the red cells are hemolyzed directly in the blood stream by the red cell antibody complement.

____ 3. May be due to overheating and infused blood exposed to extreme osmotic stress or trauma.

____ 4. This reaction, manifested by hives or urticaria, is treated with antihistamines and may be minimized by using packed red cells, reducing the dose of infused antigen.

____ 5. Produce febrile reactions due to pyrogen and toxins.

____ 6. When fever and chills complicate transfusion of platelets which have been stored at room temperature, this reaction must be primarily considered.

____ 7. This reaction may result with sudden increases in circulating blood volume not well tolerated by patients with cardiac or pulmonary disease, and anemic patients or infants.

____ 8. This reaction may be provoked by an anamnestic immune response.

____ 9. These reactions most often occur as late as 10 to 11 days and have caused acute renal failure.

____ 10. Anti-hemolytic factor (AHF) concentrates, plasma, and fibrinogen may be responsible for transmission of this disease with the risk proportional to the number of donors involved.

____ 11. Incidence of the carrier state of this disease is higher in commercial blood donors than in voluntary blood donors.

____ 12. About 6 to 12 percent of donors have leukocytes with the infection.

____ 13. Since refrigeration has a bactericidal effect, transfusion of this disease does not appear to be a serious risk in transfusion therapy today.

____ 14. Since no practical laboratory tests are presently available to exclude infective donors, a careful history must be relied upon to prevent the transmission of this disease.

Column B

a. Delayed hemolytic reactions.
b. Immediate hemolytic transfusion reaction.
c. Circulatory overload.
d. Viral hepatitis.
e. Malaria.
f. Syphilis.
g. Cytomegalovirus (CMV) infection.
h. Bacterial reactions.
i. Anaphylactic and allergic reactions.
j. Nonimmune hemolysis.

240. Indicate whether given statements correctly reflect guidelines for warming of blood.

Warming of Blood. Blood products, except platelets and thawed cryoprecipitate, should be stored in a regulated blood bank refrigerator until immediately before transfusion. If blood cannot be administered within 30 minutes, return the unit to the blood bank. Do not place blood in the ward refrigerator or near a cold window, since freezing and subsequent thawing will cause hemolysis. It is not necessary to warm the blood before administration except in unusual circumstances such as (1) massive transfusions or when the infusion rate is greater than 50 ml/min; (2) occasionally in exchange transfusion of the newborn; (3) patients with potent cold agglutinins.

If blood must be warmed prior to transfusion, this should be performed by passing the blood through coils immersed in water bath or dry incubator where the temperature is maintained at approximately 35° C, but always less than 38° C. Hemolysis may occur when blood is subjected to temperatures greater than 40° C. Blood should never be warmed by placing it near a radiator, heater, or stove. Microwave instruments are available for warming blood but serious hemolysis remains a problem. If any warming device is utilized, it should undergo careful and continuing quality control procedures and the staff should be thoroughly trained in its operation. A blood warming device which is not of the in-line type may be used only if it is located in or under the control of the blood bank. Once blood is warmed, it cannot be returned to refrigeration for future transfusion.

Exercises (240):

Indicate whether each of the following statements is true (T) or false (F). If you indicate false, explain your answer.

T F 1. Blood products, except platelets and thawed cryoprecipitate, should be stored in a regulated blood bank refrigerator until immediately before transfusion.

T F 2. In the absence of a refrigerator in the area, blood may be placed near a cold window to maintain low temperatures.

T F 3. It may be necessary to warm blood of patients with potent cold agglutinins.

T F 4. If blood must be warmed prior to transfusion, the temperature is maintained at approximately 35° C, but always less than 38° C.

T F 5. Blood may also be warmed by placing the unit near a radiator, heater, or stove.

T F 6. Once blood has been warmed it may be returned to refrigeration for future transfusion.

241. Cite the reasons for investigation of suspected transfusion reactions, the procedures to be followed in terms of their order of precedence and significance, and the most common errors resulting in hemolytic transfusion reactions.

Investigation of Suspected Transfusion Reactions. Any type of reactions, whether immediate or delayed after blood transfusion, should be reported in detail to the blood bank. It is the duty of the laboratory technician to investigate each report. Suspected hemolytic transfusion reactions must be investigated according to the method outlined in AFM 160-50, *Technical Methods and Procedures of the American Association of Blood Banks.* Since the great majority of reported adverse effects are not suspected hemolytic reactions, deviations from this protocol are acceptable, but should be spelled out in writing by the hospital blood bank director as a part of his procedure manual. Alternatively, he may evaluate each report of adverse effect personally. For example, urticarial reactions may be evaluated by simple examination of the serum for free hemoglobin and the performance of an antiglobulin test. If these are negative, the remainder of the evaluation might be omitted. Since febrile nonhemolytic reactions may mimic hemolytic episodes, a complete evaluation is usually indicated.

A standardized investigation procedure is essential for all reactions, even those tentatively considered as febrile or allergic. Of great importance, a reaction previously considered as trivial may later be blamed for some unfavorable consequence; thus, it is extremely wise for the blood bank to have a record that the reaction was investigated. Remember that the early signs and symptoms of hemolytic reaction are highly variable, and what appeared at first to be a very mild reaction may later turn out to be serious.

Schedule of Investigation. The most serious reactions involve incompatibility in the ABO system, and more than usual, the error is one of identification rather than laboratory technique. Remember that the two most deadly errors are (1) improper identification of the recipient blood samples, because the sample taken from one patient has been identified with the name of another, (2) and infusion of blood into the wrong recipient. In both cases, all laboratory procedures may be perfectly executed so *there is no excuse for such errors.*

A form documenting the investigation of suspected transfusion reaction should be used. The form should include all or some of the following information, if applicable:

1. Recheck for clerical errors.
2. Specimens needed:
   a. Pre-transfusion blood of recipient.
   b. Post-transfusion blood of recipient.
   c. Pilot samples of donor blood.
   d. Blood from container implicated in the reaction.
   e. Post-transfusion urine.
3. Immediate evaluation:
   a. Examine for visible hemolysis in the post-transfusion serum sample compared with pre-transfusion serum sample.
   b. Check urine for color—red or *coke* colored.
   c. Direct antiglobulin test—examine microscopically.
   d. Repeat ABO—on all samples.
   e. Repeat Rh—on all samples.

4. Definitive steps:
   a. Repeat major and minor crossmatch with pre- and post-transfusion samples.
   b. Repeat antibody screening, using pre- and post-samples.
   c. Special techniques as necessary:
      (1) Microscopic examination of negative reactions.
      (2) Prolonged incubation of tests.
      (3) Enzyme techniques and other studies as indicated.
   d. Bacteriologic smear and culture of contents of container.
   e. Collaborative identification of any incompatibility or irregular antibody.

5. Last steps when applicable—clinical chemistry evaluation of post-transfusion samples:
   a. Haptoglobin (2a, 2b).
   b. Methemalbumin (2a, 2b).
   c. Bilirubin (2b).
   d. Urea nitrogen (2b).
   e. Urine examination including microscopic evalatuion:

      (1) RBCs versus free hemoglobin.
      (2) Presence of hemosiderin.

(3) Chemical evaluation of hemoglobin breakdown products.

Exercises (241):

1. What type of transfusion reactions should be reported to the blood bank?

2. Why should reactions considered febrile or allergic be investigated according to standardized procedures?

3. What are two most deadly errors that cause serious transfusion reactions?

4. What should be the first step in the investigation of transfusion reaction?

5. What specimens are needed for investigation of transfusion reaction?

6. Which specimens should be compared immediately and checked for hemolysis?

7. On which samples should the ABO and Rh be done?

8. With what specimens should the major and minor crossmatch be done?

9. With what specimens should the antibody screen be repeated?

10. If and when applicable, what two common tests are included in the chemistry evaluation?

242. State the nature, characteristics, and significance of hepatitis B-associated antigen (HB$_s$Ag).

Hepatitis B-Associated Antigen (HB$_s$Ag). One of the most important recent advances in blood banking has been the discovery of hepatitis B-associated antigen (HB$_s$Ag). The term "viral hepatitis" is used to designate hepatitis caused by two specific viruses: Type A (MS–1, *infectious hepatitis*) and Type B (MS-2, *serum hepatitis*) viruses.

Blumberg and his associates discovered Australia antigen when they noted that an antibody in the blood of a transfused hemophiliac patient reacted with the antigen which had been found in the serum of an Australian aborigine. This discovery was a step forward in the understanding and differentiation of viral hepatitis, since the antigen proved to be an important marker which distinguished between the two major types of viral hepatitis, hepatitis A and B.

The Nature of HB$_s$Ag. Subsequent studies by means of electron microscopy have shown that the serum HB$_s$Ag is constantly associated with spherical particles about 20 nm in diameter, tubular forms of similar width and the Dane particle (a large particle which is about 42 nm in diameter). Actually, the exact nature of hepatitis B virus is unknown.

Not all patients who receive blood containing HB$_s$Ag develop hepatitis. Nevertheless, the risk that they will develop hepatitis is high. Since there is a close association between the detection of HB$_s$Ag in the blood and in the transmission of type B hepatitis, failure to use accepted screening procedures for HB$_s$Ag has both medical and medicolegal implication. Even though the exact length of the incubation period is sometimes difficult to determine precisely, and even though there is some overlap in the length of the incubation period, generally Type B is associated with a longer incubation time than Type A.

Exercises (242):

1. What is HB$_s$Ag?

2. With what sort of particles is HB$_s$Ag related?

3. What risk is there that a patient who receives HB$_s$Ag will develop hepatitis?

4. Which type of hepatitis is associated with a longer period of incubation?

243. Identify the methods for detection of hepatitis B-associated antigen in terms of principles, procedures, and sensitivity.

Tests for HB$_s$Ag. A variety of methods are available for detection of HB$_s$Ag, including the agar gel diffusion test (AGD), counterelectrophoresis (CEP) methods, the complement fixation (CF) test, reverse passive hemagglutination (RPHA), and reverse passive latex agglutination (RPLA) test.

Let us summarize the methods for detecting HB$_s$Ag:

Agar gel diffusion (AGD). Agar gel diffusion was the first test used for detection of HB$_s$Ag. It is simple and economical but slow. Also, it is not sufficiently sensitive for routine blood bank use.

Complement fixation (CF). The complement fixation test is said to have the same degree of sensitivity as counterelectrophoresis. The test is not well suited for some samples that contain HB$_s$Ag and HB$_s$Ab (antibody) because the antigen and antibody may form complexes that render the sample anticomplementary. The test is cumbersome and requires training and expertise.

Reverse passive hemagglutination (RPHA). This test has gained much usefulness in terms of degree of sensitivity. Red blood cells are coated with HB$_s$Ab. In the presence of HB$_s$Ag, the cells will be agglutinated.

Latex agglutination. This test is rapid and simple. It appears slightly more sensitive than CEP. Latex particles are coated with HB$_s$Ab and mixed with the test sample. If HB$_s$Ag is present, agglutination will occur. A significant percentage of false positives have been encountered, especially with serum from fresh blood samples that have not completely clotted. As a screening method, this test shows its greatest potential.

Counterelectrophoresis (CEP). When antigen and antibody are placed into adjacent wells in an agar plate, these constituents diffuse rapidly. At the interface of the two areas of diffusion, a precipitin band may be seen. When a current is passed across the two wells, the constituents move toward each other, because of an endosmotic effect on the antibody molecules, and the charge of the antigen particles. The use of the current decreases the time required for formation of a band, and increases sensitivity.

This test is commonly used to detect HB$_s$Ag in donor or recipient serum, using a reagent anti-HB$_s$Ag. Many commercial kits are available for HB$_s$Ag testing by counterelectrophoresis. Detailed instructions are supplied by each manufacturer. They should be carefully followed because the system may be affected by factors such as pH, ionic strength, temperature, time, voltage, and proportion of antigen to antibody.

The test is postive when a precipitin line is seen between the reagent antibody and the unknown serum. Controls using known weakly and moderately reacting HB$_s$Ag positive specimens should be used with each plate.

Radioimmunoassay. Radioimmunoassay (RIA) is the most sensitive test for HB$_s$Ag. The commonly used method is the *sandwich* technique. A tube is previously coated with HB$_s$Ab. The HB$_s$Ab (antibody) used is licensed by the FDA.

The donor serum sample is added to this tube and incubated at room temperature. If HB$_s$Ag is present, it will bind to the antibody on the wall of the test tube. After incubation, the tube is emptied and washed. Then ^{125}I labeled *antibody* is added and allowed to incubate. This labeled *antibody* will link to the HB$_s$Ag that has previously been bound to the unlabeled antibody on the wall of the test tube. Thus a sandwich is created, with HB$_s$Ag (if present) between labeled and unlabeled antibody. The tube again is emptied and washed, and the residual radioactivity is measured. This is compared with a control to determine if HB$_s$Ag is present.

Commonly Used HB$_s$Ag Tests. Two most commonly used methods for detecting HB$_s$Ag are radioimmunoassay and counterelectrophoresis. In one study, more confirmed positives were detected with a *third generation hepatitis test*, such as Ausria® II–125, than with counterelectrophoresis (CEP).

The following criteria are used for evaluating hepatitis tests:

a. First generation—lack sensitivity; antigen detected but not antibody.

b. Second generation—more sensitivity; antigen detected but not antibody.

c. Third generation—increased sensitivity; antigen and/or antibody detected.

The Ausria–125 (RIA) and Auscell™ (RPHA) are considered as third generation tests. Although the exact significance of the increased detection of HB$_s$Ag by the RIA method is not completely known, it is known that a number of patients who are negative by the CEP method but positive by means of the RIA technique are infectious, in that they can potentially transmit hepatitis.

In a recent Blood Bank Inspection Checklist and Report, the FDA recommends that "Each unit of blood must be tested for HB$_s$Ag on a sample of blood taken at the time of donation. The HB$_s$Ag test must be performed by either the RIA or RPHA test method if it is a routine collection. However, if blood must be used before either of the above tests can be completed, then either the CEP or reverse passive latex agglutination (RPLA) test method may be used to issue the blood, but the RIA or RPHA test must also be performed thereafter."

Exercises (243):

Match each of the following methods for detection of hepatitis B-associated antigen (HB$_s$Ag) and criteria for evaluating of hepatitis detection tests with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

____ 1. The first test used for detection of HB$_s$Ag.
____ 2. Simple, economical, but slow method.
____ 3. Not well suited for some samples that contain HB$_s$Ag and HB$_s$Ab because the antigen and antibody may form complexes that render the sample anticomplementary.
____ 4. Red blood cells are coated with HB$_s$Ab. In the presence of HB$_s$Ag, the cells will be agglutinated.
____ 5. A significant percentage of false positives have been encountered with this method especially with serum from fresh blood samples that have not completely clotted.
____ 6. In this method, when antigen and antibody are placed into adjacent wells in an agar plate, these constituents diffuse radially. At the interface of the two areas of diffusion, a precipitin band may be seen.
____ 7. The method uses ^{125}I labeled antibody; a sandwich is created with HB$_s$Ag, if present, between labeled and unlabeled antibody.
____ 8. Two most commonly used methods for detection of HB$_s$Ag.
____ 9. Classification when the test has increased sensitivity; antigen and antibody are detected.
____ 10. The RIA and RPHA method are classified accordingly.
____ 11. The agar gel diffusion method may be classified accordingly.
____ 12. A number of patients who are negative by the CEP method but positive by this method are infectious, in that they can potentially transmit hepatitis.
____ 13. According to recommendation by the FDA, either of these methods must be used to test blood for HB$_s$Ag in routine collection.
____ 14. This method or the reverse passive latex agglutination (RPLA) test may be used if the blood must be used before either the RIA or RPHA is completed.

Column B

a. Complement fixation (CF).
b. Latex agglutination (LA).
c. Radioimmunoassay (RIA).
d. First generation hepatitis test.
e. Third generation hepatitis test.
f. Agar gel diffusion (AGD).
g. Reverse passive hemagglutination (RPHA).
h. Counterelectrophoresis (CEP).

3-4. Quality Assurance in Blood Banking

The purpose of quality assurance in the blood bank or transfusion service is to insure to the greatest extent a quality product, perfectly compatible blood or blood component.

Interpretations or conclusions in blood banking are mainly subjective evaluations rather than mathematical or chemical analyses and are influenced by methodology, material, and human variables.

You will review the manner in which a Quality Assurance Program is established to monitor continually the variables mentioned.

244. State the reason for the need for quality assurance in blood banking and cite the means through which quality assurance is achieved by the blood bank technician.

The Need for Quality Assurance. The need for quality work is greater in blood banking than any other departments of the laboratory. For example, if there is an error in chemistry or hematology, while the patient simultaneously presents a more normal clinical picture in many instances, an unfortunate consequence is the integrity of the technician involved, and undoubtedly a request for repeat of the test.

Erroneous results in blood bank testing, on the other hand, can produce immediate, delayed, or even fatal results, with no second chance available. The areas for strict control are divided into (a) human factors or variables, (b) physical equipment, and (c) reagents and solutions.

Human Factors. Policies and procedures must be clearly stated in the blood bank manual of operating procedures. All methods should be kept up to date, evaluated periodically, and studied before changing a technique. In addition, all technicians must adhere to the methods and follow directions and methods provided by each manufacturer.

A current roster of personnel assigned to the department must be maintained with initials signed by each technician performing the work.

All technicians must adhere to established techniques. There must be uniformity in handling test tubes. All tubes should be observed for hemolysis. All tubes must be properly read—microscopically, or with some sort of magnification when reading final results of the Coombs test. There must be standard techniques for washing the red cells and standard interpretation of test results. For example, what constitutes a weakly reactive or a negative? A chart graphically depicting gradation of agglutination (weakly reactive up to 4+) should be available for all technicians to aid in uniform interpretation of results. In accordance with current Air Force directives, the typing and crossmatching should be double checked and countersigned by a person familiar and current with the detailed techniques and procedures being utilized.

With regard to blood storage and distribution, be sure to designate someone responsible for recording thermometer readings and changing the charts of 7 day recorders. The procedure manual should reflect a method for rotating the blood units in storage provided for economical use and unnecessary outdating.

Standard operating procedures should have directions in case the temperature alarm rings—especially after duty hours. Instructions should include the telephone number of the medical equipment repair technician on call; and if necessary, the location of alternate storage in case of equipment failure.

Proficiency testing programs from associations, such as AABB and CAP, or Government agencies should be obtained periodically. These programs evaluate the accuracy and methodology of the blood bank and compare proficiency in relation to other participating laboratories.

Internal proficiency programs, as part of a continuing education program, should be prepared utilizing weakly reactive antibodies and red cells of unusual phenotype. All technicians should participate and the results reviewed and discussed with each individual.

Exercises (244):

1. To emphasize the need for quality assurance, why must errors not be allowed in blood banking?

2. How should methods be monitored?

3. When reading final results of the Coombs test, how must all tubes be read?

4. What is suggested to aid in the uniform interpretation of agglutination?

5. In accordance with Air Force directives, how should the typing and crossmatching be accomplished through quality assurance?

6. What two instructions should be included in the procedures to follow when the blood bank alarm rings after duty hours?

7. What purpose do proficiency testing programs serve?

8. What is suggested for internal proficiency programs as a part of continuing education?

245. Indicate whether given statements correctly reflect the procedures for maintaining and monitoring the efficiency of blood bank equipment.

Equipment Control. Accuracy of testing is dependent upon properly functioning equipment. Instruments which must be properly maintained and monitored are centrifuges, Rh viewboxes, if used, water baths, incubators, and blood bank refrigerators. Each item of temperature-regulated equipment must be checked on the day of use to insure that it is functioning properly, and a record of temperature must be kept. A log for each piece of equipment should be kept including:

(1) Instrument name and serial number.
(2) Date installed.
(3) Telephone number of medical equipment repair.
(4) Date, nature of service, and name of servicing technician.
(5) Date of next servicing.

Centrifuges. For serologic testing, the most critical functions of centrifuges are enhancing red cell antigen-antibody reactions *in vitro* and washing red cell suspensions. Two types of table top centrifuges are in common use. One has a fixed speed with a variable time; the other has both variable speed and time. The time of centrifugation includes the time of acceleration, but not deceleration.

Aside from the necessary preventive maintenance checks, routine control checks of revolution per minute (RPM) and time of centrifugation are mandatory. The force created by the spinning of the centrifuge head is the relative centrifugal force (RCF) or G force. This is determined by the speed or RPM and the radius of the centrifuge head. The formula for deriving RCF is:

$$G = 0.00001118 \times RPM^2 \times R(\text{radius})$$

RPM can be determined by instruments such as the strobe light, or Jaquet, worm gear or vibrating reed tachometers. The radius of the head should be measured in centimeters from the center spindle to the outer tip of the test tube in cup. By using this formula, directions stating G forces and time required can be applied to any centrifuge.

Additional factors to be considered are: (1) Most centrifuges have an automatic timer which, after continuous use, is frequently not in alignment. The timer should be checked with a stop watch and the correction marked on the face of the timer. (2) The time of acceleration and deceleration varies from one centrifuge to another. (3) The viscosity of the medium affects the sedimentation of red cells; a greater force or longer time is required to sediment red cells suspended in 30 percent albumin than in saline.

Each centrifuge should be calibrated empirically to find the speed and time that will produce clearly recognizable agglutination, but will not cause false positive reactions. Centrifugation must be of sufficient force to bring the cells together, but not so great that the unagglutinated cells cannot be resuspended with gentle manipulation.

Calibration of the Centrifuge. Each centrifuge should be calibrated when purchased and after adjustments or repairs. Periodic monitoring, for example, every 3 to 6 months, may be done by checking the RPM and the accuracy of the timer. All observations should be part of the quality control record, and the optimum speed and time of centrifugation should be marked on each centrifuge.

It should be remembered that packing in a centrifuge is a factor of speed and time of centrifugation. Time is a more difficult parameter to control since it must be varied according to the viscosity and volume of the suspending medium through which the cells must pass in the tube.

It is, therefore, necessary to calibrate the centrifuges for each kind of test (saline, albumin, antiglobulin, enzyme) and for each particular step—washing, final readings, etc. Rechecking for control purposes must necessarily be performed in the same manner.

When the proper setting for centrifugation is found for the various media, the supernatant fluid will be clear, the cell button will be clearly delineated, and it will be readily resuspended with only very gentle agitation. Consistency in readings is essential. The final time selected must also show the strongest reaction with the positive cell while giving a clear-cut negative reaction with the negative cell. A procedure for calibration is included in AFM 160-50, *Technical Methods and Procedures of the American Association of Blood Banks.*

Water Baths, Heat Blocks, and Incubators. For detection of warm antibodies, the usual temperature of incubation is 37° C. Either water baths or heat blocks may be used; large bacteriologic type, hot air incubators are not generally recommended because of evaporation. It takes about 5 minutes longer to bring suspensions of red cells and serum to 37° C in a dry bath than in a water bath, and this time should be added to the total incubation time.

The temperature of each unit should be checked on the day of use and the results recorded. It may be difficult to determine the temperature accurately because of hot or cold spots not detected by a mounted thermometer. These areas may be detected in a water bath by positioning a thermometer in different areas. In a dry bath, hot or cold spots can be detected by a small thermometer immersed in water in 10 x 75 mm test tubes. Areas that are either too hot or too cold may be marked and avoided; preferably the unit should be repaired or replaced. Water baths with a circulating pump maintain a more even temperature, but are expensive.

Rh View-Boxes. To achieve a 37° C temperature for an Rh slide test, the surface temperature of the view-box should be 45-50° C. A special bulb is required and must be the same as that supplied by the manufacturer. It usually takes about 30 minutes to achieve optimal temperature. The light should be left on during the working day to insure that the temperature is optimal at the time testing is done. There may be a variation in temperature over the surface; different areas should be checked by a thermometer laid directly on the surface and the area of optimal temperature clearly marked. A record of the temperature on the day of use should be recorded.

Thermometers. The accuracy of, and agreement between, all thermometers used to monitor laboratory devices must be verified. Accuracy may be checked using a National Bureau of Standards certified thermometer or a fever thermometer which is obtainable in degrees C. The manufacturer of fever thermometers is more closely regulated than laboratory thermometers.

All new thermometers should be checked before using. Older thermometers should be rechecked during routine preventive maintenance operations.

Exercises (245):

Indicate whether each of the following statements is true (T) or false (F). If you indicate false, explain your answer.

T F 1. Each item of temperature regulated equipment must be checked at least 2 days after each use to insure proper functioning; a record of temperature must be kept.

T F 2. Time of centrifugation includes both time of acceleration and deceleration.

T F 3. Routine control checks of RPM and time of centrifugation are mandatory.

T F 4. The relative centrifugal force (RCF) or G force is the same as the RPM.

T F 5. The radius of the head should be measured in centimeters from the center spindle to the outer tip of the test tube in cup.

T F 6. The timer need not be checked.

T F 7. A lesser force or shorter time is required to sediment red cells suspended in 30 percent albumin than in saline.

T F 8. Each centrifuge need not be calibrated since the speed and time can be used for all similar brands in the blood bank.

T F 9. Packing in a centrifuge is a factor of speed and time of centrifugation.

T F 10. Speed is a more difficult parameter to control since it must be varied according to the viscosity and volume of the suspended medium through which the cells must pass in the tube.

T F 11. When the proper setting for centrifugation is found for the various media, the supernatant fluid will be clear, the cell button clearly delineated, and it will resuspend readily with only very gentle agitation.

T F 12. It takes about 10 minutes longer to bring suspensions of red cells and serum to 37°C in a dry bath than in a water bath.

T F 13. In a dry bath, hot or cold spots can be detected by a small thermometer immersed in water in 10 x 75 mm or 12 x 75 mm test tubes.

T F 14. To obtain a 37° C temperature for an Rh slide test, the surface temperature of the view box should be 55 to 60° C.

T F 15. It is not necessary to check new thermometers before using.

246. State the requirements for maintaining quality control of blood bank reagents such as antiserum controls, reagent red blood cells, antihuman globulin serum, and bovine albumin.

Blood Bank Reagents. Before any degree of reliability of test results can be obtained, reagents must be monitored for quality and dependability. While regulation and licensure of commercial reagents by the Bureau of Biologics of the Food and Drug Administration help to insure initial integrity, it is the responsibility of each laboratory to continue the quality controlling of such licensed products.

Since the Bureau of Biologics has no jurisdiction over the condition under which antiserums or reagent cells are delivered to the laboratory, this check is in the hands of the personnel in the blood bank. Today very few clinical transfusion services make any of their own reagents. While the potential for preparing almost any blood bank reagent does exist in many laboratories, medicolegal problems dictate the use of products from reputable sources licensed by the Bureau of Biologics of the Food and Drug Administration. The Standards require that reagents used routinely should be tested periodically during use to demonstrate reactivity. Antiserums must be able to detect weakly reactive antigenic determinants; test cells must be able to detect weak antibodies. Quality control should reflect the extent of serologic testing of each institution and will be influenced by the volume of work and the type of service; for example, blood bank or transfusion service. Certain procedures are applicable in evaluating reagents when received or when put into use; others are better adapted for periodic evaluation of reagents during use. Red cells and antiglobulin reagents are more likely to deteriorate than antiserums such as anti-A or anti-B.

Quality control should be practical and realistic. It should give evidence that reagents are reactive and specific on the day of use, but should not be so extensive as to be excessively time consuming or accumulate unnecessary data. If reagent red cells are used to test antiserums, two controls can be done with one test. However, if reactivity is diminished, both antiserum and test cells must be tested separately to identify the cause.

In all records, the date, lot number and source of antiserums and test cells, and identification of personnel should be included. When the tests are completed, the record should be reviewed to determine the significance.

Antiserum Controls. Certain commercially supplied antiserums are licensed by FDA, and each lot must meet minimum requirements for specificity, potency, and avidity. Minimum requirements have not been established for other less commonly used reagents. It is important for the user to test reagents because of possible deterioration during shipping, storage, or use. Materials may be tested when received or when a new lot is put into use, but opened vials should be tested as part of a daily control program. For example, it may be desirable to titer each reagent when received, but to do either an avidity or a dilution test on the day of use for confirmation of reactivity.

To prevent deterioration, antiserums should be stored at 1° to 6° C when not in use. If frozen for extended storage, they should be thawed at 37° C and thoroughly mixed before use. They should not be thawed and refrozen repeatedly; rare antiserums may be divided into aliquots before freezing.

The procedures used must reflect the directions of the manufacturer. The manufacturer's directions should be periodically reviewed for changes.

Reactivity. Reactivity of antiserums is usually demonstrated by avidity, titration, and dilution rather than by testing with weakly reacting red cells, since it is difficult for most blood banks to have these phenotypes readily available.

Specificity. Specificity may be established by testing reagents with some red cells that are positive and some negative for the antigenic determinants. It should be remembered that if there is a return of unwanted anti-A or anti-B in an antiserum during storage, it will not be detected by group O red cells.

Infrequently used antiserums. Infrequently used antiserums should be tested with positive and negative controls on the day of use. When possible, weakly reacting red cells should be selected for the positive control. If red cells from a panel of group O cells are used, one that is heterozygous for the antigenic determinant should be selected.

Avidity testing. Avidity is the speed and intensity with which an antigen and antibody react. The test is done on a slide and is observed macroscopically for agglutination. ABO and Rh antiserums (Modified Tube and Slide Test Reagent) can be tested by this method. Results should be recorded on a quality control log. Method of testing for avidity of anti-A and anti-B is included in AFM 160-50, *Technical Methods and Procedures of the American Association of Blood Banks.*

Titration procedure. The titer of serum is the reciprocal of the highest dilution of serum giving a 1+ agglutination. In calculating the titer, the dilution caused by the addition of the red cell suspension is excluded.

Dilution procedure. A single dilution of antiserum may be made for testing with appropriate red cells for periodic quality control in accordance with minimum requirements established by FDA.

Reagent Red Blood Cells. Periodic visual inspection for evidence of deterioration is recommended. Red cell suspensions showing only slight hemolysis may probably be used satisfactorily if the hemoglobin-stained supernatant can be removed by one saline wash. Those with evidence of excess hemolysis should be discarded. Because red cells may lose reactivity during shipping, storage, or use, it is important that they be tested on the day of use with weak antibodies.

Antihuman Globulin Serum. It is necessary that the reactivity of antihuman serum be tested each day of use and when a new lot of reagent is used. The minimum Federal requirements for this serum relate only to the ability of the serum to react with a weak $Rh_o(D)$. This diluted antibody must be given a 1+ reaction with antihuman serum and must also give the same titer result when the antihuman serum is diluted 1:4 with saline.

Bovine Albumin. Bovine albumin must also be tested for specificity and reliability on the day of use and when new lots arrive. Quality control testing should assure the user that the following are avoided or absent:

a. Hemolysis or darkening of red cells.
b. Crenation of red cells.
c. Rouleaux-forming properties.

Positive and negative controls are also necessary to verify the specificity of the reagent and its ability to enhance agglutination.

Exercises (246):

Complete each sentence with the appropriate word or words. A phrase rather than a single word may be required for correct response.

1. Before any degree of reliability of test results can be obtained, reagents must be monitored for ____________ and ____________.

2. The responsibility for regulations and licensure of regulations and licensure of commercial reagents is that of the ______________.

3. The responsibility for the continued controlling of these products is that of the ____________.

4. Standards require that reagents used routinely should be tested periodically during use to demonstrate ______________.

5. It is required that each lot of certain commercially supplied antiserum meet minimum requirements for __________, __________, and ____________.

6. ____________ is the speed and intensity with which an antigen and antibody react.

7. Avidity, titration, and dilution procedures are used to demonstrate the ________ of antiserums.

8. When the testing of reagents with some red cells that are positive and some that are negative, for the antigenic determinants, is accomplished, ____________ may be established.

9. Those red cell suspensions showing evidence of excess hemolysis should be ____________ .

10. The minimum Federal requirements for anti-human serum is the ability to react with a ____________ .

11. Quality control testing of bovine albumin should assure the user that (a) ____________ , (b) ____________ , and (c) ____________ are avoided or absent.

CHAPTER 4

The Blood Donor Center

"SAFE HUMAN BLOOD and blood products" might well be the ultimate goal of all blood donor services. The phrase is taken from the Federal Food, Drug, and Cosmetic Act which states that "It is the purpose of the action (Act) to encourage uniform practices in blood banks throughout the Nation, and to raise the level of such practices so that American consumers will have 'sufficient quantities of high quality, safe human blood, and blood products.' "

In recent years, requirements for shipping blood to US combat forces overseas have placed an even greater demand on military blood banks. They must not only supply their own ever increasing day-to-day needs, but must also support the military blood program in other geographic areas as directed. Blood donor center operation, and the procurement of blood routinely and during national emergencies, will be discussed in this chapter.

Despite the fact that in recent years many blood donor centers have acquired more stature and greater autonomy, they are being subjected to increased external controls especially at state and national levels. These controls call for improved blood bank organization and practice.

There are currently in the United States two nationwide blood bank inspection programs. One is conducted by the American Association of Blood Banks (AABB) and the other by the Food and Drug Administration (FDA), Public Health Service, Department of Health, Education, and Welfare. AFM 160-24, *Standards for Blood Banks and Transfusion Services,* will be used as a guide throughout this chapter.

In the final analysis, the blood donor, the one original source, must be properly selected and be in a state of good health. Even so, there remains the risk that certain disease conditions not readily apparent may be transmitted to a susceptible or weakened patient. Only the alert and resourceful blood bank technician can continue to function under such imposing restrictions by following well established guidelines for donor selection. The experience can be a safe and rewarding one for the potential recipient, the donor, and the blood bank technician.

4-1. Donor Selection and Collection of Blood

As a blood bank technician, you will be responsible for seeing that an adequate blood supply for transfusion is available. Since the only source of human blood is a human donor, the blood donor must be well treated and protected. You must apply your technical training to care for the donor and encourage him to return to donate again. By proper donor-technician relationships, the clinical transfusion service supply system can be maintained. Just as a rule in medicine is "do not harm the patient," the rule in blood banking is "do not harm the donor *or* the patient." In this section, we will describe donor eligibility and accepted techniques of drawing blood for transfusion.

247. Indicate whether given statements correctly reflect the purpose and importance of the Blood Donor Record, DD Form 572.

Blood Donor Record. You obviously cannot draw blood from just anyone who walks through the door of your laboratory. On the other hand, it is not practical to provide a complete physical examination for every blood donor. Rather, the blood banker must rely on a statement of medical history from the prospective donor and a few simple medical factors (usually temperature, pulse, blood pressure, and hemoglobin). The findings of the examination and a brief medical history are recorded on DD Form 572, Blood Donor Record. A facsimile of DD Form 572 is included in foldout 2 printed and bound in back of this volume. Before proceeding, turn to the foldout 2 and fill out DD Form 572 as if you were the donor. Also, enter what you consider to be normal data for your weight, temperature, pulse, blood pressure, hemoglobin, and hematocrit. Then, review your entries after you have read this section and determine if the information you have recorded on DD Form 572 qualifies you for donation.

Importance of the Blood Donor Record. DD Form 572 is an important blood banking record. It lists pertinent facts about the donor's health, which are useful in protecting the donor and the patient; but remember, a record of facts is by itself of limited value. It is your responsibility to interpret this information. Have you assured yourself that the donor is healthy before taking his blood? If a donor suffers a reaction, the DD Form 572 can protect you. On the other hand, i you disregarded low hemoglobin, high blood pressure, or other disqualifying factors, the blood donor record card can be incriminating indeed! Remember, the rule of selecting the healthy donor should not be *stretched* for reasons of expediency or to avoid hurting a would-be donor's feelings.

Here are some additional ways in which the blood donor record card is of value:

a. DD Form 572 tells you something about a unit of blood. It is, in a sense, the medical history of the unit.

b. The donor record card permits followup. It tells you how often the donor gave blood at your facility. If a patient develops hepatitis or any other transfusion-related condition, necessary followup action can be taken. This may include treating the donor or removing his name from the donor list. When information concerning the health of a donor requires, it is your responsibility to make this known to the Environmental Health Services, or other appropriate unit of the hospital. When in doubt, consult your supervisor.

c. The form tells you who collected the blood. This can be of some value, especially if the unit becomes contaminated or in some way unsatisfactory.

d. DD Form 572 helps identify the unit because it bears an accession number, which also appears on the unit.

Exercises (247):

Indicate whether each of the following statements is true (T) or false, (F) and correct those that are false.

T F 1. The findings of the medical examination and a brief medical history are recorded on DD Form 573.

T F 2. The Blood Donor Record pertains to facts about the donor's health, which are primarily useful in protecting the donor and the technician.

T F 3. It is the responsibility of the laboratory technician to initially interpret the information on the Blood Donor Record.

T F 4. The Blood Donor Record may be considered a medical history of a unit of blood.

T F 5. If a patient develops hepatitis, for example hepatitis, type B, it is the donor's responsibility to relay this information to Environmental Health Services since he is a carrier of the disease.

T F 6. If several units drawn were found to be contaminated, DD Form 573 or SF 518 may be used to identify the technician(s) who collected these units.

T F 7. The DD Form 572 bears the transfusion number which helps to identify it.

248. Cite the criteria for selection of blood donors in terms of data obtained from the physical examination.

Donor Criteria. Let us now turn our attention to specific eligibility criteria. A medical officer should approve all blood donors, and sign DD Form 572 in the space provided. Authoritative guidelines for donor criteria are available in AFM 160-24, *Standards for Blood Banks and Transfusion Services* and AFM 160-50, *Technical Methods and Procedures of the American Association of Blood Banks.*

A limited physical examination and a rather detailed medical history must be done on the *day of each* donation to determine whether giving blood will in any way harm the donor or if transfusion of the unit will in any way harm the recipient.

The order in which questions are asked or examinations performed may be arranged for convenience. The interview must be conducted by qualified personnel, as designated by the medical director, in a manner that assures privacy as indicated, allays apprehensions, and allows time for any necessary discussion or explanation. Results of tests must be recorded. Answers to questions should be recorded as *Yes* or *No* with details added as indicated.

Physical Examination. The required procedures with some acceptable methods and allowable parameters for acceptance, deferment, or permanent rejections are presented in the paragraphs that follow.

Weight. Donors weighing 110 pounds (50 kg) or more may ordinarily give 450 ± 45 ml of blood in addition to pilot tubes not exceeding 30 ml. Donors weighing less than 110 pounds may be bled proportionately less in a reduced volume of anticoagulant. A history of recent excessive weight loss may indicate further questioning.

Temperature. The oral temperature shall not exceed 37.5° C (99.6° F.). *Caution:* Glass thermometer should not be in mouth when finger is pricked.

Pulse. The pulse should exhibit no pathologic irregularity and be between 50 and 100 beats per minute.

Blood pressure. The systolic blood pressure shall be between 90 and 180 mm of mercury and the diastolic should not exceed 100 mm of mercury. Prospective donors with diastolic pressure between 90 and 110 mm of mercury or with abnormal differences between diastolic and systolic pressure (*examples:* 115/90, 180/70) should be investigated. The blood bank physician must evaluate any question of heart disease revealed in the medical history as well as abnormal pulse or blood pressure findings before a donor can be accepted.

Skin lesions. The skin at the site of venipuncture shall be free of lesions including stigmata of drug injection. Examine both arms.

Mild skin disorders such as acne, psoriasis, or the rash of poison ivy are not cause for deferment unless in an anticubital area. Donors with boils or other severe skin infections should be deferred.

General appearance. If the donor looks ill or appears to be under the influence of drugs or alcohol or is excessively nervous, it is best to defer temporarily.

Hemoglobin or hematocrit. The preferred method is determination of hemoglobin concentration. The hemoglobin shall be no less than 12.5 gm per 100 ml for female donors and no less than 13.5 gm per 100 ml for male donors. The hematocrit value, if substituted, shall be no less than 38 percent for females, and no less than 41 percent for males. Hemoglobin may be estimated by the copper sulfate method or determined by spectrophotometric methods.

Exercises (248):

1. What two authoritative references contain guidelines for selection of blood donors?

2. What two reasons are given for a limited physical examination and a detailed medical history?

3. What is the minimum weight at which a donor may give 450 ± 45 ml of blood in addition to pilot tubes not exceeding 30 ml?

4. What should be the acceptable pulse in addition to showing no pathologic irregularity?

5. What is the acceptable range for the systolic and diastolic blood pressure?

6. Under what conditions should donors with boils or severe skin infections be allowed to donate?

7. From your observations of the general appearance of the donor, if he looks ill, or appears to be under the influence of drugs or alcohol or excessively nervous, what should be your best course of action?

8. Would you reject a female donor with previous history of successive donations whose present hemoglobin is 11.9 gm per 100 ml? Why?

249. Indicate whether given statements of medical history correctly reflect the criteria for acceptance, deferment, or permanent rejection of blood donors.

Medical History. Some very specific questions will be necessary, but a great deal of pertinent information can be obtained by asking some general or leading questions in simple language that the donor can understand. The examples given below include all requirements and are followed by suggested or required response to information received.

Age. Blood donors shall be between their 17th and 66th birthdays. Donors who are less than 21 years of age are accepted if (1) they are members of the Armed Forces over 17 years of age, (2) they are civilian minors (as defined by the state) for whom written consent is given by the parent or legal guardian, or (3) they are married and over 17 years of age.

Medical questionnaire. The following questionnaire may be used as a guideline and for acceptance:

a. Have you ever given blood? Date of last donation? Interval between donations of whole blood must be 8 weeks except in unusual circumstances and with the written permission of a physician.

b. Have you ever been rejected as a blood donor? Why?

c. (Women) Are you pregnant? Have you been during the last 6 weeks? Defer during and for 6 weeks after termination of pregnancy. Exception may be made if possible autologous or exchange transfusion is anticipated.

d. Do you feel well now? Acute respiratory infection is cause of deferment until all symptoms subside. Symptoms such as pain, cough, sore throat, headache, nausea, dizziness, menstrual cramps, sinusitis, or extreme nervousness may be cause for deferment depending on severity and medical evaluation.

e. Are you in good health generally?

(1) Under a doctor's care now for any reason? Why?

(2) Ever had a serious illness or operation? When? What?

(3) Any illness or operation in last 6 months? When? What?

(4) Ever cough up or vomit blood? Explain.

(5) Suffer from chest pain or shortness of breath? Explain.

Donors should be in good health. This group of questions may reveal the presence or history of kidney, lung, heart, stomach, or liver disease or cancer. Be sure all are either denied or explained. Acutely ill donors must be deferred or excluded. Chronic conditions should be evaluated by a physician.

A history of rheumatic heart disease or coronary heart disease is cause for rejection. Single episodes of rheumatic fever or pericarditis, a heart murmur, or repair of a congenital defect do not necessarily disqualify a donor.

Active pulmonary tuberculosis is cause for rejection. Donors who have developed a positive tuberculin skin test, but lack X-ray abnormality, may be accepted provided they have not taken prophylactic medication in 48 hours.

Infectious mononucleosis is not cause for rejection if not associated with jaundice and recovery is complete.

Surgical procedures. Donors who have had major surgery should be deferred for at least 6 months. Minor surgery is disqualifying only until healing is complete. The determination of *major* and *minor* may have to be made by a physician, but the following common operations are generally classified as minor: closed reduction of fracture, repair of hernia, hemorrhoidectomy, appendectomy, tonsillectomy, minor gynecologic procedures, removal of pilonidal cyst, varicose veins, and dental surgery.

f. Are you taking any drugs or medications? Why? What? Marijuana (unless presently under influence), oral-contraceptives, mild analgesics, minor tranquilizers or psychic energizers, vitamins, replacement hormones, or weight reduction pills are not usually cause for deferment or rejection. NOTE: Aspirin or aspirin containing compounds depress platelet function for 12 to 18 hours. A donor who has taken these drugs within 48 hours should not be used as the *only* source of platelet preparations (plateletphoresis).

History of recent or present therapy with antibiotics, corticosteroids, digitalis, insulin, quinidine, dilantin, diuretics, nitroglycerin, anticoagulants, or other potent drugs should be evaluated by a physican.

g. Have you been vaccinated or had any shots in the past 3 months? What? When? Ever had rabies shots? When? Why?

Sympton-free donors who have been immunized with toxoids, or killed viral, bacterial, or rickettsial vaccines are acceptable after 24 hours. This includes tetanus, typhoid, paratyphoid, cholera, diphtheria, typhus, Rocky Mountain spotted fever, influenza, polio (Salk), and plague and prophylactic rabies duck embryo vaccines.

Smallpox—Donors are acceptable either after the scab has fallen off or 2 weeks after an immune reaction.

Measles (rubeola), mumps, yellow fever, oral polio vaccine, and animal serum products—Donors are acceptable 2 weeks after their last immunizing injection or ingestion.

German measles (rubella)—Donors are acceptable 3 months after their last injection.

Rabies (therapeutic)—Donors will be deferred until one year after their last injection.

h. Do you have any allergies? A history of any reaction to drugs or medications within 6 months, or presently symptomatic allergy such as asthma, hay fever, or urticaria, should be evaluated by the medical director. Prospective donors with symptomatic bronchial asthma or who have received a hyposensitization injection within 72 hours should be deferred.

i. Have you had convulsions or fainting spells? Donors who have had either except in childhood may have a reaction or seizure if they donate.

j. Do you bleed a long time if you have a cut or have had a tooth pulled? Abnormal bleeding tendency is cause for rejection. Physician should evaluate.

k. Have you ever had malaria?

(1) Are you a visitor or immigrant to the USA?

(2) Have you been out of the USA in the past 6 months? Where?

(3) Have you ever taken any medication to prevent malaria?

Travelers in areas considered endemic for malaria by the Malaria Program Center for Disease Control, US Department of Health, Education, and Welfare, may be accepted as regular blood donors 6 months after return to a nonendemic area providing they have been free of symptoms and have not taken antimalarial drugs. Prospective donors who have had malaria shall be deferred for 3 years after becoming asymptomatic and after cessation of therapy. Prospective donors who have taken antimalarial prophylaxis or who have been military personnel in an endemic area shall be deferred for 3 years after cessation of therapy or after departure from the area if they have been asymptomatic in the interim. Immigrants or visitors from endemic areas may be accepted as blood donors 3 years after departure from the area if they have been asymptomatic in the interim. Donations to be used for the preparation of plasma, plasma components, or fractions devoid of intact red blood cells are exempted from these restrictions.

l. Have you ever had yellow jaundice or hepatitis? Have you been exposed to hepatitis in the past 6 months?

(1) Have you had close contact with a patient with the disease?

(2) Skin graft?

(3) Tatoo?

(4) Ear piercing?

(5) Blood or plasma given to you?

(6) Ever injected any drugs into your veins or your skin?

m. Have you ever had a positive test for syphilis? Ever been treated for syphilis? A positive serologic test for syphilis is cause for rejection. Donors may be acceptable when they become seronegative, provided the previous positive result was not due to a condition which would result in continued exclusion.

Exercises (249):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. Married females under 21 must have written consent from sponsors in order to donate blood.

T F 2. Intervals between donations of whole blood must be 18 weeks except in unusual circumstances and with the written permission of a physician.

T F 3. Women who are pregnant are deferred during such state and for 6 weeks after termination of pregnancy.

T F 4. Symptoms such as cough, pain, sore throat, headaches, menstrual cramps, or extreme nervousness may be cause for deferment depending upon severity and medical evaluation.

T F 5. A history of rheumatic heart disease or coronary heart disease does not necessarily disqualify a donor.

T F 6. Donors who have developed a positive tuberculin skin test but lack X-ray abnormality may be excluded permanently.

T F 7. Infectious mononucleosis is a cause for rejection if associated with jaundice and recovery is not complete.

T F 8. Donors who have had major surgery should be deferred until healing is complete.

T F 9. Oral-contraceptives, mild analgesics, minor tranquilizers, vitamins, or weight reduction pills are not usually cause for deferment or rejection.

T F 10. A donor who has taken aspirin or aspirin containing compounds may be used as the only source of plateletphoresis.

T F 11. Donors showing no symptoms, immunized with toxoids or killed virus, bacterial or rickettsial vaccines such as tetanus, cholera, influenza, and polio are acceptable after 24 hours.

T F 12. German measles (rubella) donors are acceptable 3 months after their last rejection.

T F 13. Abnormal bleeding tendency may help in the processing of collection and is *not* a cause for rejection.

T F 14. Prospective donors who have taken antimalarial prophylaxis or who have been stationed in an endemic area shall be deferred for 3 years after cessation of therapy.

T F 15. A Vietnamese doctor from an area considered endemic for malaria arrived in the States in January 1975. He wanted to donate blood in January 1977. He should be accepted as a donor providing he meets all other criteria satisfactorily.

T F 16. Receiving plasma, skin graft, tattoo, ear piercing, or injection of any drugs into the veins or skin may be considered as exposure to hepatitis.

250. State the criteria for permanent exclusion or deferment of donors with questionable history of viral hepatitis and on donations by flying personnel.

Viral Hepatitis. The possible presence of the agent of viral hepatitis cannot at present be detected with certainty by any available means including history, physical examination, or laboratory tests (including tests for HB_sAg). Therefore, strict regulations for donor acceptability must be established and followed.

a. Exclude permanently:

(1) Donor with a history of viral hepatitis at any time.

(2) Donor if his was the only unit of blood, blood component, or derivative administered to a recipient who within 6 months developed post-transfusion hepatitis. NOTE: Post-transfusion hepatitis after multiple transfusions is not cause for exclusion of all donors.

(3) Donor who is or has been a drug addict (involving injection of drugs). Check *both* arms.

(4) Donor who has ever had a confirmed positive test for HB_sAg.

b. Defer for at least 6 months (in case hepatitis develops):

(1) Recipient of blood, blood components, or derivatives such as fibrinogen, Factor 2,7,9, 10 complex, AHG concentrates, and immune vaccines. This includes donors who are in blood group immunization programs.

(2) Skin allografts, tattoo, ear piercing.

(3) Donor who has had close contact with a patient with viral hepatitis. The type of contact hospital personnel encounter in their routine work is *not* considered close contact and is not cause for exclusion. Hospital personnel in areas where hepatitis is endemic (in renal dialysis units, etc.) should probably be excluded for 6 months after employment in such areas.

(4) Inmates of penal or mental institutions until 6 months after release.

Any donor with a questionable history or one who has been implicated in more than one case of post-transfusion hepatitis should be referred to the blood bank physician for evaluation.

Record of physical examination and medical history must be signed by the examiner. The reason for deferral or rejection should be explained to the donor and referral to a physician made if indicated.

Donors who are accepted should be made aware that there is possible risk to the recipient and asked to report any illness developing within a few days of donation and especially to report hepatitis developing within 6 months.

Restrictions on Donations by Flying Personnel. In accordance with AFR 160-26, *Air Force Blood Program,* air crews of high performance or combat aircraft and persons occupying cockpit positions in an on-call status to perform essential flight duties will not donate blood.

Exercises (250):

1. When is a donor with a history of viral hepatitis deferred?

2. For how long is a donor who is or has been a drug addict deferred?

3. When is a donor who has ever had a confirmed positive test for HB_sAg deferred?

4. Why is there a deferral for at least 6 months for donors with skin allografts, tattoo, and ear piercing?

5. Under what conditions will personnel on flying status be permitted to donate blood?

251. State some basic prerequisites for collection of donor blood, and the type of containers used for blood collection in terms of their purposes and the type of anticoagulant.

Collection of Blood. Blood shall be collected from donors by trained personnel working under the direction of a qualified, licensed physician. The donor room should be well lighted, at a comfortable temperature, clean, and pleasant.

Blood collection shall be by aseptic methods, utilizing a sterile, closed, or vented system, and a single venipuncture. Vented systems shall employ bacterial air filters. If more than one skin puncture is needed, another container and donor set must be used. The donor must never be left unattended during the collection of blood.

Containers. The standard blood collecting container consists of a plastic bag with an integral plastic tube attached, as shown in figure 4-1,A. The blood pack unit shown is available for collection of 450 ml of blood in 63 ml of Citrate Phosphate Dextrose (CPD) Solution U.S.P. Plasma, cells, or a portion of the blood may be transferred from the blood collecting bag to a transfer pack as shown in figure 4-1,B. Cells may be separated from plasma by centrifugation or gravity and considered as packed red cells. A plasma extractor may be used in the process of separating the plasma from the cells.

Integrally connected double, triple, or quadruple plastic containers with completely closed systems are used to permit salvage of blood components or of fractional blood units. These units may be used in transfusions of small children and babies. An example of a double blood pack with one 300 ml transfer pack for such purposes is shown in figure 4-2.

The plastic bag is composed of an inert polyvinyl plastic, which is less damaging to thrombocytes and cells than a glass surface.

Figure 4-1. (A) Blood collecting bag and (B) transfer pack.

The needle is usually a 16 gauge, stainless steel phlebotomy needle firmly attached to the plastic tubing. Once the needle cover is removed, the needle should be used at once because it cannot be considered sterile if the cover is replaced. The donor tube is imprinted with a repeating series of numbers so that it can be sealed into identified segments for laboratory use. The tube also contains CPD. A stainless steel bead is positioned at the juncture of the tube and the bag. The bead acts as a valve to keep in the anticoagulant.

Unused bags from an opened can may be kept 30 days by securing in polyethylene pouch to prevent possible loss of moisture.

Exercises (251):

Complete each sentence with the appropriate word or words. A phrase rather than a single word may be required for the correct response.

1. Blood shall be collected from donors by trained personnel working under the direction of a qualified ____________________.

2. If more than one skin puncture is needed another ____________ and ____________ must be used.

3. CPD stands for ____________ ____________ ____________ solution, the anticoagulant in th standard collecting container.

4. Cells may be separated from plasma by ____________ and ____________.

5. Plasma, cells, or portions of the blood may be transferred from the blood collection bag to a ____________________.

6. The plastic bag is composed of an inert polyvinyl plastic, which is less damaging to ____________ and ____________ than a glass surface.

7. Once the needle cover is removed, the needle should be used ____________________.

8. The stainless steel bead in the bag acts as a ____________ to keep the anticoagulant.

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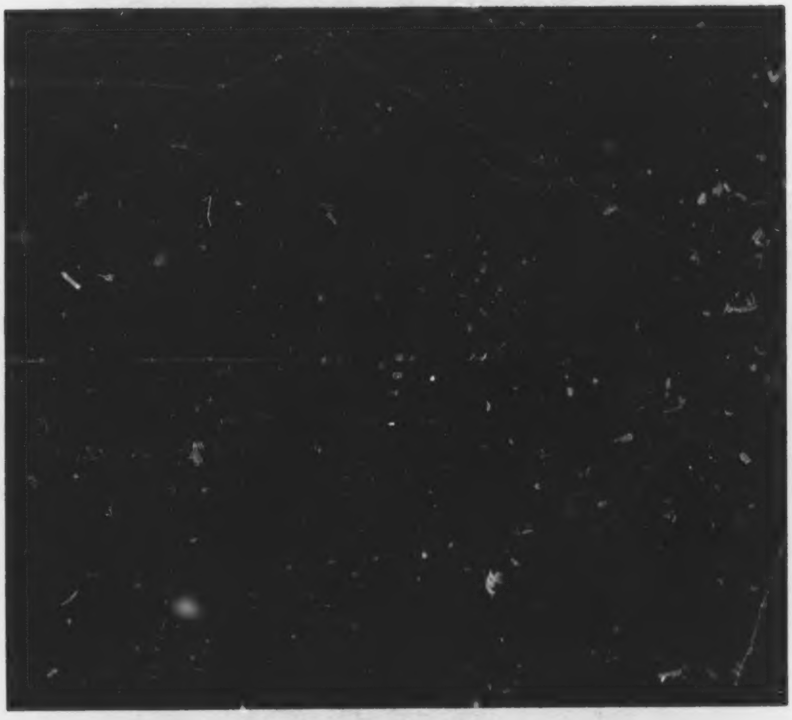

Figure 4-2. Double blood-pack unit with one 300 ml transfer pack.

9. Unused bags from an opened can may be kept for ____________ days by securing in polyethylene pouch to prevent possible loss of ____________ .

252. Point out the given devices used for blood collection, state how they operate, and cite specific ways of preparing the donor for blood donation.

Blood Collecting Apparatus. When setting up the container for blood collection, you may use either a plastic spring scale often used for blood collection in mobile units, blood collection balance, or a Hemolator. The collecting balance shown in figure 4-3 has the advantage of providing an automatic shutoff if the donor tube is strung through as shown. Occasionally, the donor tube pinches off if it is not placed correctly through the cutoff arm. This restricts the free flow of blood. Unless a vacuum chamber is used, the bag should be hung well below the level of the donor's arm. A Hemolator, which is used for vacuum assisted blood collection into plasma bags, is shown in figure 4-4. The Hemolator, in operation, agitates the bag as bleeding proceeds. The Hemolator serves as a mechanical means for assisting in the collecting of blood from the donor into the blood bag unit. It is designed to accelerate collection by application of negative pressure to the exterior of the blood bag unit. Automatic agitation serves to mix the blood and anticoagulant during collection. When the predetermined quantity has been collected, the flow of blood into the blood bag unit is automatically stopped. The Hemolator operates with a vacuum pump not shown.

Donor Preparation. Be sure that the label on the bag matches the donor number on DD Form 572, Blood Donor Record, and on any additional pilot tube that may be used.

Before you position the donor on the table or in the donor chair, review his record card and be sure that he is eligible to give blood. Then you should examine both of the donor's arms to select the arm with the best vein. Sometimes you must apply a tourniquet or blood pressure cuff before you can make this determination; but do select the better arm before proceeding with a skin prep. When you have decided which vein you wish to use, position the donor. If a table is used, do not use a pillow because this elevates the person's head and may contribute to vertigo (dizziness). Try to make the donor comfortable and avoid contributing to any mental anxiety he may have. A calm professional manner is in order. Casual conversation is acceptable, but do not tell the patient he has *bad veins* or otherwise suggest that he may have difficulty. The phlebotomist who repeatedly asks such questions as "Are you sure you feel all right?" is psychologically encouraging the donor to react.

At this point, the donor is comfortable, and all of your equipment is in place. The room should be about

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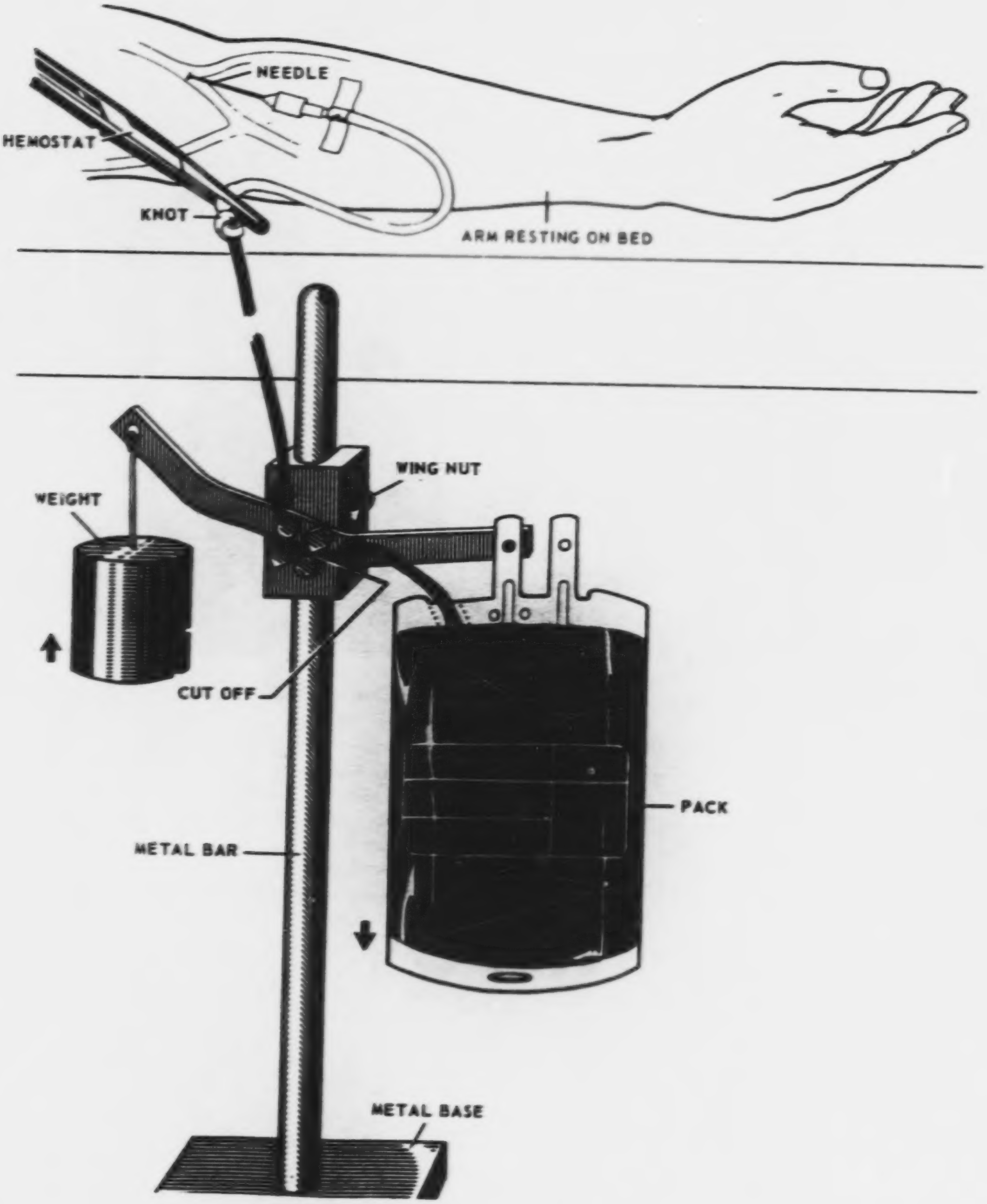
NEEDLE
HEMOSTAT
KNOT
ARM RESTING ON BED
WING NUT
WEIGHT
CUT OFF
PACK
METAL BAR
METAL BASE

Figure 4-3. Blood collecting balance.

72° F (22.2° C) and well ventilated. It is better to keep the temperature cool rather than overly warm. Female donors should be covered with a sheet over their legs. Keep the donor area clean and free from blood stains. Though spattered blood does not bother an experienced technician, it can be repulsive to the average person. Finally, before you begin to draw the blood, be certain that a physician is in the building and can be summoned quickly. At least one other person must be within calling distance so that you can summon aid if the donor develops a reaction.

Exercises (252):

1. What three devices may be used for blood collection?

Figure 4-4. Hemolator for automatic mixing and vacuum assistance of blood collection.

2. How does the collecting balance shut off automatically?

3. How does the Hemolator operate in blood collection?

4. What should you do before you position the donor on a table or in the donor chair?

5. If a table is used, why should a pillow NOT be used?

6. Why is it NOT a good policy to ask the donor such questions as, "Are you sure you feel all right?" or "Let me know if you don't feel well."?

7. What two things should you make certain of before you begin to draw?

253. Indicate whether given statements correctly reflect the procedure for preparation of venipuncture for blood donation.

Venipuncture Preparation. Select a large, firmly attached vein in an area free from skin lesions. It is often helpful to inspect both arms and to use either a tourniquet or a blood pressure cuff inflated to 50-60 mm mercury to make the veins more prominent. Having the donor open and close his hand a few times is also helpful. Release the tourniquet and prepare the site.

There is no practical way to prepare an aseptic site for venipuncture; however, a state of surgical cleanliness can be achieved. The suggested cleansing of the phlebotomy site includes the following:

a. Scrub vigorously with surgical soap for at least 30 seconds with gauze to clean away fat, oils, and dirt. Swab the skin in a rotary fashion, holding the 2 x 2 gauze pad or cotton with a forceps and moving from the intended venipuncture site to the periphery. Scrub with firm pressure and repeat until the 2 x 2 pad no longer appears *dirty* before using the alcohol and iodine.

b. To remove soap, apply 10 percent acetone in 70 percent alcohol and allow to dry.

c. Apply tincture of iodine (3 percent in 70 percent alcohol) and allow to dry.

d. Remove the iodine with 10 percent acetone in 70 percent alcohol. (The iodine has served its purpose and will rarely cause any skin reactions if properly removed.) Allow the solution to dry.

e. Cover site with dry sterile gauze unless ready to do venipuncture. NOTE: Keep the tincture of iodine bottle tightly capped to prevent evaporation of alcohol. Higher concentrations of iodine may cause skin reactions. Do not use mercurials or quaternary ammonium solution which may be readily neutralized by skin proteins. It is very important to clean the site thoroughly to prevent contamination of the unit or infection of the donor. As we mentioned, handle gauze and cotton pads with a forceps. Some technicians have the very bad habit of using their fingers to apply the pads, and what is worse, squeezing excess solution back into the container of antiseptic. This act rinses bacteria from their hands into the stock container. (Remember, most so-called antiseptics do not eradicate bacteria immediately upon contact. *Staphylococcus* organisms can survive for long periods in 70 percent alcohol.) Remember, after cleansing the skin, do not touch the venipuncture site unless you swab it again.

Donor Reaction. Occasionally, a donor develops a convulsive-like reaction, and you need to be prepared. If this reaction does occur during the phlebotomy, remove the needle and protect the donor from biting his tongue by using a tongue depressor. Prevent the donor from falling off the table or suffering other injury. Usually, elevation of his feet, a cool towel on his forehead, and other simple measures will resolve the problem, but the physician in attendance should be consulted.

Exercises (253):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. Alcohol is used in the first step of cleansing the phlebotomy site to remove fat, oils, and dirt.

T F 2. Swab the skin in a rotary fashion, holding the gauze with a forceps and moving from the periphery to the intended site of the venipuncture.

T F 3. Remove the iodine with 10 percent acetone in 70 percent alcohol.

T F 4. Mercurials or quaternary ammonium solution may be readily neutralized by skin proteins.

T F 5. Squeezing excess solution back into the stock container of antiseptic is a good technique for preventing waste of antiseptic.

T F 6. After cleansing the skin, do not touch the venipuncture site unless you swab it again.

T F 7. If a donor develops a convulsive-like reaction, continue the drawing of blood until the convulsion stops.

254. Indicate whether given statements correctly reflect the procedure for obtaining a clot-free collection of donor blood.

Phlebotomy. You are probably familiar with the phlebotomy technique. In order to obtain a clot-free full collection of blood, it is most important to use a large bore needle and that there be a clean, skillful venipuncture.

To refresh your memory, let us review some of the steps in starting the flow of blood.

a. Inspect the bag for any defects. The anticoagulant in the bag must be clean.

b. Carefully set the bag in position. If the balance system is used, be sure that the counter-balance is adjusted for the amount to be drawn. Unless metal clips and a hand sealer are used, make a *very loose* overhand knot in tubing. Hang the bag and route tubing through the pinch clamp, as noted in figure 4-3. If the balance system is not used, position the bag low enough to allow gravity collection. It may be hung upside down so blood flows through the anticoagulant. If using a Hemolator, certain modifications are necessary, and the manufacturer's instructions should be followed.

c. Reapply the tourniquet or blood pressure cuff. Have donor open and close hand until previously selected vein is again prominent.

d. Uncover the sterile needle and do venipuncture immediately. If desired, tape the tubing to hold needle in place. Cover site with sterile gauze.

e. Release the bead at the base of the donor tube to permit blood flow into the collecting bag. Squeeze the tubing above the bead between thumb and forefinger to force the bead completely free into the blood pack.

f. Have donor open and close hand, squeezing a rubber ball or other resilient object, slowly and continuously during collection.

g. As soon as the blood flow has been established, mix the blood and anticoagulant gently and continuously during collection. Mixing may be done manually, by placing bag on a rotator, or by using a Hemolator.

h. Blood flow will stop after the proper amount has been collected when using the balance or vacuum-ssist methods. Otherwise, the bag must be weighed (spring scales) and the flow stopped manually.

i. Seal the tubing 4 to 5 inches from the needle by making a white knot or using a metal clip.

j. Grasp tubing on the donor side of the seal and press to remove blood for a distance of no more than an inch. Clamp with hemostat.

k. Cut tubing between seal and hemostat and collect desired pilot samples.

l. Deflate and remove tourniquet. Remove needle from arm. Apply pressure and have donor raise arm (elbow straight) and hold gauze firmly over phlebotomy site with other hand.

m. Discard needle assembly into special container.

n. Starting at seal, strip donor tubing as completely as possible into the bag. It is important to work quickly before initiation of coagulation occurs.

o. Invert bag several times to mix thoroughly; then allow tubing to refill with anticoagulated blood from the bag.

p. Tubing is left attached to the bag and may be sealed into sterile segments suitable for crossmatching using knots, metal clips, or a dielectric sealer. A final double seal should be made within two inches of the bag.

q. Recheck numbers on container, pilot tubes, and donor record.

r. Blood must be either refrigerated or processed for components immediately. Red cells must be refrigerated as soon as possible.

Exercises (254):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. The first step before phlebotomy is to inspect the bag for any defects.

T F 2. Unless metal clips and a hand sealer are used, make a very tight knot in the tubing.

T F 3. If the balance is not used, position the bag at arm level.

T F 4. After uncovering the sterile needle, the venipuncture may be delayed up to 1 hour.

T F 5. If spring scales are used, the flow of blood stops automatically.

T F 6. After the proper amount is obtained, seal the tubing 4 to 5 inches from the needle by making a white knot or using a metal clip.

T F 7. A cut should be made between the seal and hemostat to collect desired pilot samples.

T F 8. Stripping of donor tubing may be delayed or omitted since coagulation is delayed by anticoagulant.

T F 9. Blood must be either refrigerated or processed for components immediately.

T F 10. Delayed refrigeration of red cells is permissible.

4-2. Blood Storage and Shipment and the Air Force Blood Program

There has been a great deal of progress made in the storage and preservation of blood, both in the liquid form or frozen. Some technicians may feel that the question of red cell storage and preservation is not their concern; that it can be left to the manufacturer of blood containers, or to the research workers. This is far from correct. Daily, the blood bank technicians make decisions that should be influenced by some knowledge of red cell enzymology and principles of blood storage. The technician is often required to participate in decisions concerning the question as to whether a patient really needs fresh blood, the choice of CPD over ACD, and the choice of citrate solutions over heparin.

The human erythrocyte is a living cell that requires energy, usually derived from glucose, to remain viable and carry out its function of delivering oxygen to the tissues. Since the major purpose of transfusing whole blood and red cells is to provide the recipient with the means of delivering oxygen to the tissues, hemoglobin function as well as viability must be considered during the storage period.

Finally, we will describe the significant features of the Air Force Blood Program in terms of the goals to provide whole blood and certain blood products in peacetime and for treatment of military casualties during national emergencies, mobilization, or conflict.

255. Identify the breakdown of constituents that occur during liquid storage and cite the purpose and properties of given preservatives used in the storage of blood.

Breakdown During Liquid Storage. On storage a series of events take place. In brief, the red cell membrane loses lipid. The integrity of the sodium-potassium pump is adversely affected. Potassium leaks from the red cells, sodium enters, and equilibrium between the two ions is approached. Even though the process is retarded by low temperatures, eventual breakdown products of glycolysis, notably lactic acid and accummulate, occur and eventually the pH falls. The falling pH tends to inhibit enzymes necessary for the phosphorylation of glucose. Since this is the first step in glycolysis, glucose metabolism gradually ceases. Adenosine triphosphate (ATP) is also necessary for the phosphorylation of glucose, and without it no energy metabolism of the erythrocyte is possible.

In addition, the red cells become depleted of 2,3-diphosphoglycerate (DPG). This depletion results in an increased affinity of the red cell hemoglobin for oxygen, which in turn lowers the capacity of the transfused blood to deliver oxygen to the recipient's tissues. Possibly DPG-depleted red cells should not be given to patients under stress.

Acid-Citrate-Dextrose (ACD) and Citrate-Phosphate-Dextrose (CPD). The blood preservatives ACD (acid-citrate-dextrose) and CPD (citrate-phosphate-dextrose) allow the red cell to maintain concentrations of adenosine triphosphate (ATP) to assure adequate viability for 21 days of storage at 4° C. The slight difference in volume of two preservatives is of no concern. The amount of preservative required for the quantity of blood to be collected is present in the containers when they are sterilized. In both of these preservatives, there is more than enough citrate ion to bind calcium in the plasma, thus preventing clotting. The low storage temperature has an inhibiting effect on certain red cell enzymes controlling the glycolytic metabolism of dextrose so that concentrations of dextrose are maintained during storage at a level which gives adequate viability of the red cell for at least 3 weeks. The maximum allowable storage time is referred to as *shelf life*. This time is determined by the requirement that at least 70 percent of the transfused red cells must remain in the circulation 24 hours after transfusions. Transfused red cells which survive 24 hours will subsequently disappear from the circulation at a normal rate.

ATP concentrations are initially maintained better in ACD because the lower pH of this preservative favors maintenance of ATP. However, later in storage ATP concentrations are frequently higher in CPD-stored blood. This is probably because of the two millimolar (mM) phosphate present in CPD which contributes to the adenosine phosphate pool. There is a good correlation between ATP levels and post-transfusion viability of stored cells.

The 2,3-DPG concentrations are clearly better maintained in CPD-stored blood throughout the 3-week storage period because of the higher pH of CPD-stored blood. Hemoglobin function expressed as p50 (an inverse function of oxygen affinity) is maintained at near normal values for around 2 weeks in CPD-stored blood, whereas the p50 in ACD-stored blood has decreased to values below normal during the first week of storage. Periodic mixing of the blood throughout the period of storage appears to maintain higher levels of glucose, ATP, and 2,3-DPG throughout the storage period.

Heparin. Heparin is a natural anticoagulant which has been used for collecting blood for cardiopulmonary-bypass surgery and for exchange transfusions. Heparin activates lipoprotein lipase, producing an increase in circulating free fatty acids. Since these substances compete with bilirubin for binding sites on albumin, the advantages of heparinized blood must be weighed carefully against this disadvantage when choosing an anticoagulant for blood for neonatal exchange transfusions. Because it lacks dextrose and has a higher pH which stimulates glycolysis, heparin serves only as an anticoagulant and not as a preservative. Blood collected in this solution must be used within 48 hours. Heparin is not recommended for routine blood collection.

Exercises (255):

1. In the process of breakdown in storage, what substance is lost from the red cell membrane?

2. What happens to the potassium and sodium?

3. When the pH falls, what other essential process of red cell metabolism is affected?

4. What other substance is necessary for the phosphorylation of glucose?

5. When 2,3-diphosphoglycerate (DPG) is depleted, how is the red cell hemoglobin affected?

6. How is the transfused blood affected by increased affinity of the red cell hemoglobin for oxygen?

7. What is the given purpose of ACD and CPD as preservatives?

8. How is shelf life determined?

9. What is the probable cause for a higher concentration of ATP noted later in CDP-stored blood as compared to ACD-stored blood?

10. Why is the 2,3-DPG concentrations better maintained in CPD-stored blood throughout the 3-week storage period than in ACD?

11. What two reasons are given for the use of heparinized blood?

12. What is the maximum allowable time for use of blood collected in heparin?

256. State the requirements for routine storage of blood and blood storage refrigerators.

Routine Storage of Blood. Blood storage refrigerators should contain only blood, blood components, reagents for blood bank tests, and blood samples from patients and donors. All samples in test tubes should be stoppered. The temperature must be maintained in all areas of the refrigerator between 1° and 6° C with no fluctuation of more than 2° C within this range. Blood storage refrigerators should be designed with adequate fans and circulating spaces so that the designated temperature is maintained throughout the refrigerator.

Separate areas in blood storage refrigerators should be clearly designated and labeled for: (1) unprocessed blood, (2) labeled blood, (3) crossmatched blood, (4) rejected or quarantined blood. Separate shelves or areas should be labeled for the various blood types and groups.

Blood should be stored in a refrigerator that is coupled to a temperature recording device and provided with an alarm system, which is activated if the proper temperature is not maintained. The thermostat controlling this alarm must be a double point system, registering temperatures both above and below the range. The alarm system should sound in an area where some responsible person is on duty 24 hours a day. If this person is not part of the blood bank staff, he must be briefed on what to do if the alarm sounds. Alarm systems must be battery operated. Batteries equipped with trickle chargers are available for this purpose.

Temperature charts from 7-day mechanical recording devices must be changed weekly, dated inclusively, and labeled for proper identification of the refrigerator. Any temperature variation from normal should be explained in writing on the chart beside the tracing; but if the tracing is habitually a perfect circle, the recorder may not be functioning properly. The person responsible for changing the charts should sign them. All temperature records should be kept as part of the permanent blood bank records.

Blood must not be stored in refrigerators on wards or other places where the refrigerators do not meet all of the criteria for a blood bank refrigerator. Remember that blood storage refrigerators are used only for blood, blood products, typing serums, pilot tubes, or reagents directly related to blood banking. Never use a blood bank refrigerator for storing bacteriology media, food, reagents from hepatitis B associated antigen testing, or any material not related to blood banking. Only specially designed commercial blood bank refrigerators should be used. Outdated blood must be promptly removed from the blood bank refrigerator so that it will not be inadvertently administered to someone.

Exercises (256):

Complete the following statements:

1. Blood storage refrigerators should contain only ________, ________, and ________ for blood bank tests and ____________ from patients and donors.

2. The temperature must be maintained in all areas of the refrigerator between _____° C and _____° C with no fluctuations of more than _____° C.

3. Blood should be stored in a refrigerator that is coupled to a ________ ________ device and provided with an ________ ________.

4. Temperature charts from the mechanical recording devices must be changed ________, dated inclusively, and labeled for proper identification of the ________.

5. All temperature charts should be kept as a part of the ________ blood bank record.

6. ________ blood must be promptly removed from the blood bank refrigerator so that it will not be erroneously given to someone.

257. Describe the process of cryopreservation of red blood cells and cite the clinical usefulness for red blood cell cryopreservation and storage.

Cryopreservation. Red blood cells to which an appropriate amount of a cryoprotective agent such as glycerol solution has been added may be stored at ultra-low temperatures for long periods of time. Prior to transfusion they are thawed and the glycerol is removed by a washing technique. Freezing arrests the metabolism of the cells, while glycerolization-deglycerolization removes the plasma and the great majority of the nonerythrocytic formed elements.

Glycerol was first used as the cryoprotective agent for red cells over 25 years ago by Smith. The application of this discovery was largely developed in the laboratories of three principal investigators. Tullis, working with the US Naval Hospital, Chelsea, Massachusetts, developed a glycerilizing procedure based upon the continuous flow centrifugal apparatus of Cohn. Huggins developed a noncentrifugal method whereby cells were suspended in nonelectrolyte solutions and sediment to the bottom of the container, enabling the decanting of the supernatant glycerol. Rowe used a lower concentration of glycerol with rapid freezing and storage in liquid nitrogen to minimize the deglycerolization-washing process.

The development of techniques by these researchers for successfully freezing and thawing of red cells has been one of the greatest advancements in blood banking since the institution of ACD and subsequently CPD preservatives.

Clinical Usefulness. Freezing red blood cells for indefinite lengths of time for subsequent transfusion has become an accepted blood banking procedure which may solve some blood bank inventory problems. Platelet concentrates, fresh frozen plasma, and cryoprecipitates may be removed from a unit before freezing the red cells, providing optimal use of each unit of whole blood.

Frozen red cells may eliminate or minimize the clinical problems associated with post-transfusion hepatitis, transfusion of red cell antibodies, tissue antigens present on white cells and platelets, plasma protein antigens and antibodies, leukocyte-borne viral disease, hyperkalemia, and anticoagulant toxicity.

Red cells stored for longer than 21 days can be treated with buffered nutrients to restore normal levels of metabolites before preparation for frozen storage. The unused nutrients are subsequently removed by the washing process. These rejuvenated red cells have the same post-transfusion survival and viability as red cells which were frozen on the day of collection from the donor. Red cells which have been continuously stored at below -80° C do not lose 2,3-diphosphoglycerate.

Other Clinical Considerations. Although frozen red cells have numerous advantages over conventionally stored blood, a major disadvantage is the greater expense due to the processing. It is these economic considerations which have prevented their wider use. Nevertheless, there are other indications for use.

Renal dialysis units. Frozen deglycerolized blood has two advantages over liquid banked blood for dialysis patients, in that the infectivity of hepatitis and HL-A antigenicity are reduced.

Organ transplantation. Since it is almost leukocyte free, cryopreserved blood is useful in patients awaiting or undergoing organ transplantation.

Patient with leukocyte antibodies. More than 90 percent of the leukocytes and platelets may be removed in the process.

Patient with antibodies to plasma antigens. Extensive washing removes the plasma, whereas the IgA-deficient patient who has reactions to blood or blood components containing IgA benefits immensely from the use of cryopreserved blood.

Patient with rare blood types. For elective procedures or in emergencies, it is often possible to secure rare blood types from banks that maintain frozen blood.

Patient undergoing open heart surgery. Cryopreservation has the advantages of eliminating plasma antibodies and microemboli in donor blood.

Exercises (257):

1. Briefly describe the basic process of cryopreservation.

2. What purpose does freezing of the cells serve?

3. What is accomplished by glycerolization-deglycerolization?

4. What components may be removed from each unit before freezing?

5. List some of the clinical problems that may be eliminated or minimized by frozen red cells.

6. What two advantages does frozen deglycerolized blood have over liquid banked blood for dialysis patients?

7. How does the IgA-deficient patient who has reactions to blood or blood components containing IgA benefit from the use of cryopreserved blood?

8. What advantages does cryopreservation have for patients undergoing open heart surgery?

258. Identify the methods used for the cryopreservation of red cells, and cite the procedures and the advantages or disadvantages of each.

Method of Cryopreservation. There are two general methods of freezing blood: (1) high-glycerol (approximately 40 percent W/V), slow-freezing with storage at -80° or -85° C, and (2) low glycerol (approximately 20 percent W/V), rapid-freezing with storage at -150° or -196° C. The method of choice is dependent upon the equipment available in the blood transfusion laboratory.

High-glycerol slow-freezing. One apparatus developed for the high-glycerol, slow-freezing method is the Cytoglomerator™. In devising this equipment, Huggins took advantage of the fact that red cells in a low-salt, low-pH medium will agglomerate into large masses. This agglomeration leads to rapid sedimentation and eliminates the need for centrifugation in washing and deglycerolizing the red cells. Centrifugation is still required for the removal of plasma and for adjusting the final red cell concentration.

Procedures are subject to change with the development of improved techniques, but according to current practice, the blood is collected in standard bags containing ACD or CPD. Within 6 days after collection, the plasma is removed, and a high-glycerol solution is introduced. The solution also contains glucose, fructose, and ethylenediamine tetra-acetic acid (EDTA). The EDTA prevents positive antiglobulin tests due to the low ionic strength of the medium. If CPD is the anticoagulant, a small amount of citric acid also is added. The glycerolized cells are frozen in special long, thin plastic bags at -80° C. When required, the red cells are removed from the freezer and thawed in a waterbath at about 37° C within 7 to 10 minutes. Deglycerolization is accomplished by first adding 500 ml of 50 percent glucose to the red cells. They are then washed twice with 2000 ml of 5 percent fructose. The red cells agglomerate and sediment rapidly in the low-electrolyte medium; thus, deglycerolization is rapidly accomplished. At the end of the washing procedure, the cells are suspended in 250 ml of physiological saline solution. This disperses the agglomerates. The resuspended red cells are centrifuged and dispensed with a hematocrit of 75 to 85 percent. The deglycerolized cells must be used within 24 hours after removal from storage.

The high-glycerol methods using continuous flow or semiautomated batch washing are probably the most popular at the time of this writing. While the capital outlay for heavy equipment may be high, the personnel time involved in their operation is minimal and the conditions of storage are somewhat less rigid. The cell recoveries are high, and the product is becoming less expensive.

Low-glycerol rapid-freezing. The low-glycerol rapid-freezing method was developed with the intent to simplify the freezing procedure and to shorten the post-thaw washing time with a minimum of cell injury and cell loss. Blood is collected in standard bags containing ACD or CPD. Within 5 days of collection, the plasma is removed and a solution of glycerol, sodium chloride, and mannitol is added to the packed cells. The mixture is frozen in liquid nitrogen. Storage is in the gaseous phase of liquid nitrogen at -150° C or with liquid nitrogen at -196° C. Because of the low temperatures involved, the low-glycerol method has been recommended for storage periods greater than 2 years. However, blood processed by the high-glycerol method has been stored successfully for many years at -80° C.

Thawing is rapidly accomplished in a 45° C waterbath and the thawed cells are deglycerolized. Most users of the low-glycerol method employ one of the commercially available washing devices. The wash solutions were formerly mannitol-saline, but a newer method is to use saline solutions. Deglycerolization, with saline and a cell washer, can be accomplished in as little as 15 minutes.

Cells prepared by this method are of excellent quality, and the percentage of recovery is good. The low-glycerol method is comparably rapid and simple, but as indicated, requires ultra-low temperature systems maintained by liquid nitrogen. Liquid nitrogen may be expensive in some regions, and storage and transportation vessels are also cumbersome and expensive.

Exercises (258):

1. What are the two general methods for freezing blood?

2. What is the storage temperature of both methods?

3. **What principle of red cell conditions did Huggins utilize in devising the use of the Cytoglomerator™?**

4. What does this agglomeration by Huggins principle lead to?

5. For what purposes is centrifugation still required in this procedure?

6. What constituent in the high-glycerol solution prevents positive antiglobulin tests due to the low ionic strength of the medium?

7. How is deglycerolization by this method first accomplished?

8. What is the hematocrit reading after the final centrifugation and dispensing of the red cells?

9. In the low-glycerol, rapid-freezing method, what solution is added to the packed cells after separation of the plasma?

10. In what constituent is the mixture frozen?

11. Despite the fact that the low-glycerol method is comparably rapid and simple, what could be some disadvantages?

259. Indicate whether given statements correctly reflect the procedural guidelines for transportation of blood and frozen components.

Shipment and Transportation of Blood. Blood must be kept between 1° and 10° C during shipment. Double wall cardboard boxes or cardboard boxes with specially designed styrofoam inserts are used. Examples are shown in figure 4-5. The boxes are clearly labeled "Human Blood". Never ship blood in a container labeled otherwise. The best way to ship blood any distance is by air. Ordinary ice is used, not dry ice. The ice is placed in one or two plastic bags and tied securely. Ideally, the ice pack should not touch the blood bags directly, because this increases the chances of damage and hemolysis. Shipment and complete instructions concerning transportation of blood are found in AFR 168-3, *Operational Procedures for Military Blood Donor Centers and Armed Services Whole Blood Processing Laboratories;* additional guidelines are given in AFM 160-50, *Technical Methods and Procedures of the American Association of Blood Banks,* and AFR 160-26, *Air Force Blood Program.*

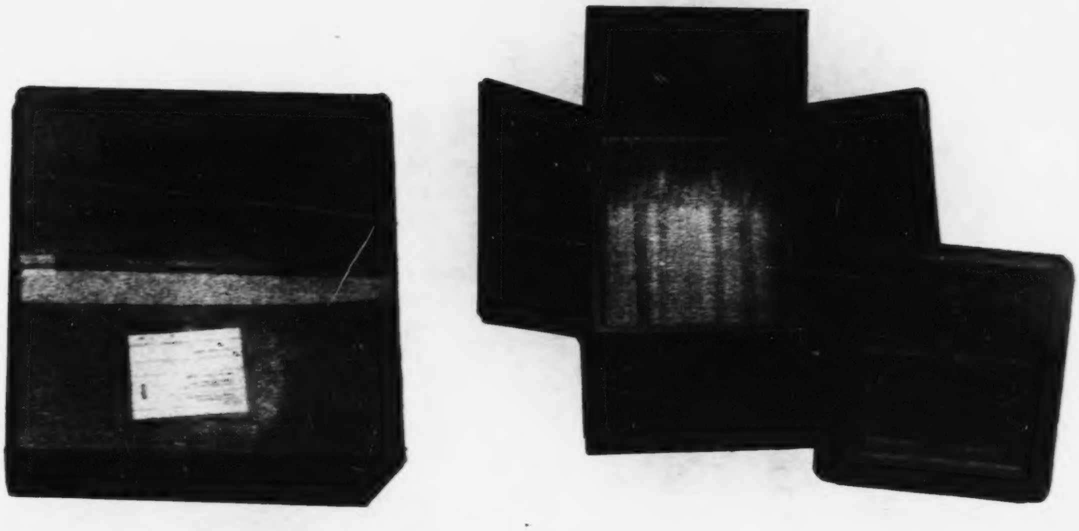

Figure 4-5. Blood shipping container.

Shipment. A shipping record is a necessary part of transporting blood. Use DD Form 573 (Shipping Inventory of Blood Collections) when shipping blood through DOD blood program channels. DD Form 573 or a similar form is usually required when blood is shipped to private blood banks or other facilities. Keep the following points in mind:

a. If you ship and receive blood, you must account for each unit.

b. Pay particular attention to expiration dates. If a unit of crossmatched blood is to be returned to a lending blood bank, it may be necessary to either release the unit or crossmatch another unit. Blood that is not shipped on time is usually *charged off* to the facility concerned. Blood is too expensive and difficult to obtain to permit waste! Even more important, a medical problem arises if expired blood is held for a patient. Obviously, you cannot give expired blood.

c. The DD Form 573 is a valuable document. It provides continuity in identifying the units with other records at the collecting and receiving centers. The form may also help trace the blood or establish liability if a shipment is lost in transit. A copy of DD Form 573 is printed in foldout 2.

Transportation. Blood should not be released to the carrier prior to 1 hour before its departure. If the scheduled train, bus, or plane departure is delayed after the blood has been released to the carrier, the carrier should contact the transportation officer. To insure shipment, it has often proved necessary for a representative from the blood bank to remain with the blood until it has departed. It is also wise to notify the recipient by AUTOVON or other rapid means when a shipment is scheduled to arrive.

Blood containers should never be exposed to extreme temperatures. Blood must not be placed in the lower compartment of planes or in any place where the ambient temperature falls below 34° F. If there is delay enroute, the carrier may be instructed to break the seal in order to re-ice. If this is done, a signed statement from the carrier agent who breaks the seal, indicating the time and date re-iced, must be placed in the container for the receiver's information. Wet ice *only* is used for reicing. (Dry ice lowers the temperature too much.) The receiver must not accept a container of blood with a broken seal unless the explanation is signed by the carrier. In such a case, the receiver should notify the officer in charge of the donor center immediately.

Blood must be transported as expeditiously as possible. Every attempt must be made to have blood at its destination the same day it is shipped. Processing laboratories (for oversea blood shipments) receive blood 24 hours a day; therefore, the local carrier should not hold containers of blood overnight for delivery the next day.

Transportation of Frozen Components. Packing must assure that the component being transported is maintained at or below its required storage temperature. For example, if fresh frozen plasma (required to be stored below minus 20° C) is to be packed for a 10-hour trip, enough dry ice must be used to assure that the plasma stays below minus 20° C. The insulation qualities of a clean plastic air bubble packaging material have been useful in maintaining temperatures of frozen components and protecting them from mechanical damage. Adequate quantities of dry ice and insulating characteristics of the container must be determined for each situation.

Exercises (259):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. Blood must be kept between 2° and 4° C during shipment.

T F 2. The best way to ship blood is by Railway Express.

T F 3. Dry ice is commonly used for the shipment of blood.

T F 4. The ice pack should not touch the blood bags directly, because this increases the chances of damage and hemolysis.

T F 5. Complete instructions concerning the shipment and transportation of blood is contained in AFM 160-50.

T F 6. A shipping record is a necessary part of transporting blood, and DD Form 572 is used for this purpose.

T F 7. If a crossmatched unit of blood with a close expiration date is to be returned to the blood center, it must be held by your lab until it expires.

T F 8. The DD Form 572 provides continuity in identifying the units with other records at the collecting and receiving centers.

T F 9. Blood should not be released to the carrier prior to 1 hour before its departure.

T F 10. If the scheduled train, bus, or plane departure is delayed after the blood has been released to the carrier, the carrier should contact the chief of blood transfusion service.

T F 11. The receiver must not accept a container of of blood with a broken seal unless the explanation is signed by the carrier.

T F 12. Every attempt must be made to have blood at its destination within 24 hours after shipment.

T F 13. Clean plastic air bubble package material may be useful in maintaining temperatures of frozen components and protecting them from mechanical damage.

260. Indicate whether given statements correctly reflect the goals, functions, and organization of the DOD Military Blood Program, the Military Blood Program Office, and the Air Force Blood Program.

Department of Defense Military Blood Program. The Department of Defense blood program was organized with the essential goal to make whole blood and blood components available for the wounded serviceman, no matter what part of the world he was servicing. It was decided that the most effective organization to provide this vital product would be a jointly staffed agency with the structure and authority to coordinate the resources of the Army, Navy, and Air Force. The program provides for the maintenance of a source of trained personnel, facilities, supplies, and equipment adequate to meet all emergencies, ranging from local disasters to general war. It further provides for a research and development program devoted to progress and improvement in the field. The Department of Defense Military Blood Program encompasses the blood program of the organizations of the three military departments, the organization of the Joint Chiefs of Staff, the Unified and Specified Commands, and the Military Blood Program Office.

Military Blood Program Office (MBPO). Under present concepts, the MBPO is a triservice-staffed DOD organization which monitors the policies and decisions of the Assistant Secretary of Defense (Health and Environment) relative to the military blood program. The MBPO is responsible for coordinating and integrating the plans, policies, and procedures of the unified and specified military commands. The Assistant Secretary of Defense (Health and Environment) is responsible for providing overall policy guidance on the DOD Military Blood Program, and for coordinating it with the National Emergency Blood Program, when required.

Air Force Blood Program. Guidance for the operation of the Air Force Blood Program is contained in AFR 160-26, *Air Force Blood Program*. This regulation tells how to obtain whole blood in peacetime and during contingencies.

Peacetime blood program. Air Force medical facilities normally obtain blood for transfusions from:

a. Volunteer donors and military bases without payment.

b. Other Government medical facilities such as AF medical centers, AF regional hospitals, Armed Services Whole Blood Processing Laboratory, or other nearby Federal medical facilities.

c. The American Red Cross (ARC) in the United States and Puerto Rico (including Alaska and Hawaii) with Red Cross regional blood programs.

d. Civilian blood banks licensed by the Food and Drug Administration.

e. Purchase from individuals, at specified rates, when whole blood is not available from the sources listed above. Blood from paid professional donors, or from blood banks which pay donors will not be used. Exceptions to this rule may be made by the hospital commander in emergency or unusual geographical situations.

Donor information. Blood may be donated by military personnel, DOD personnel, and dependents of military personnel. It is a command responsibility to provide donors at the frequency and in the quantity necessary to enable Air Force hospitals to maintain a working inventory of blood in the appropriate groups and types and to meet contingency requirements for blood. Each base commander will formally designate a non-medical service officer or senior NCO as Air Force Blood Program Officer.

Exercises (260):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. The DOD blood program was organized with the prime goal to make whole blood and components available to the wounded serviceman serving in any part of the world.

T F 2. The program provides for maintenance of a source of trained personnel, facilities, supplies, and equipment adequate to meet all emergencies except local disasters.

T F 3. The program also provides for a research and development program devoted to progress and improvement in the field of blood transfusion service.

T F 4. The policies and decisions of the Assistant Secretary of Defense (Health and Environment) are monitored by the DOD Blood Program commanders.

T F 5. The MPBO is responsible for the plans, policies, and procedures of the unified and specified military commanders.

T F 6. Guidance for the operation of the Air Force Blood Program is contained in AFR 160-24.

T F 7. Military volunteer blood donors may accept payment for blood in military facilities.

T F 8. Blood may be accepted from civilian blood banks that are pending licensing by the FDA.

T F 9. Blood from paid professional donors or from blood banks which pay donors will be used.

T F 10. It is the base level responsibility that donors be provided at the frequency and in the quantity needed by Air Force hospitals and to meet contingency requirements for blood.

T F 11. Each base commander must designate a nonmedical service officer or senior NCO as Air Force Blood Program officer.

261. State the purpose of the National Blood Program and the agency responsible for its function.

National Blood Program. The National Blood Program was established to meet the Nation's requirement for whole blood, blood derivatives, and plasma in the event of mobilization or national emergency. The Office of Emergency Planning is responsible for the program. The Department of Defense insures that blood collecting facilities, distribution points, and processing laboratories are available to supply whole blood for treatment of military casualties during national emergencies, mobilization, or war.

Exercises (261):

1. What is the purpose of the National Blood Program?

2. What office is responsible for the National Blood Program?

3. What organization is responsible for blood collecting facilities, distribution points, and processing laboratories for supplying whole blood for treatment of military casualties during national emergencies, mobilization, or war?

262. Specify the operation and function of the USAF Blood Donor Center.

Military Blood Donor Centers. In response to directives of the department that military blood requirements be met under all circumstances, a very elaborate, well-organized, and efficient donor center system was developed among the three military services.

For example, all of the whole blood used during the Southeast Asia Conflict was collected from voluntary DOD blood donors, mainly active duty military personnel, their dependents, and DOD civilian employees.

Designated stateside and oversea bases maintain standby and active blood donor centers in accordance with the directives of Volume 1 of the USAF War and Mobilization Plan. Most of these active centers are located at large military training installations where most donor quotas can be adequately met. Each blood donor center must be under the control and technical supervision of the director of base medical services. They must be capable of collecting, classifying, storing, and shipping 600 units of whole blood each 30 days; except the Blood Donor Center at Wilford Hall, Lackland AFB, Texas, which will have the capability to process 3,600 units each 30 days.

The blood donor centers are organized and operated in accordance with AFR 168-3, AFM 160-50 and AFM 160-24.

Each center collects blood in quotas established by HQ USAF—using only stocklisted collecting/dispensing bags.

Blood donor processing. Blood donor centers vary somewhat according to the local situation. Bearing this in mind, let's describe a typical blood donor center.

The blood donor center is operated on an assembly line basis. Various stations are set up, and the donors proceed from one to the next. A different phase of donor blood procurement is accomplished at each. Donors are first registered in groups of 50 to 100. At this time, the donor completes the DD Form 572; and each medical questionnaire is checked by a physician. Those who are disqualified are directed to the transportation station for transportation to their point of origin, or they are dismissed. The accepted donors are directed to the physical examination station where their blood pressure, pulse, temperature, and hemoglobin are checked. Before the donor leaves this area, his DD Form 572 is checked again.

From the physical examination station, the donor proceeds to the drawing area, where he is given a cup of fruit juice. After donating, all donors are required to wait 10 to 15 minutes in a recovery area supervised by a physician and nurses. At some centers, the donor then goes to the dining hall for a post-donation meal.

Even though donors are screened and drawn in an assembly line fashion, they are treated as individuals. Time is taken to answer each question and to insure the safety and care of each donor. Each volunteer receives a donor card similar to that used by the Red Cross. The success of any blood donor center depends on willing donors. By treating the donors as individuals and showing them that we are concerned for their safety and welfare, we reap immeasurable benefit.

Blood collection and processing. In the drawing area, each unit of blood is checked for proper amount, pilot tubes, and serial number. The units are then arranged by ABO grouping and sent to the donor center's processing area. The processing of each unit includes an ABO serum grouping and Rh typing with antiserums which meet FDA standards. If the blood is typed as Rh$_o$(D) negative, it shall be tested using a technique designed to detect Rh$_o$ variants (D^u). Routine testing for additional blood types is not recommended. The label shall indicate:

a. Rh Positive—when the red cells react for Rh$_o$(D) or Rh$_o$ variants (D^u).

b. Rh Negative—when the red cells are nonreactive for Rh$_o$(D) and Rh$_o$ variants (D^u). All group O units are titered for anti-A and anti-B. Donor blood is tested for unexpected antibodies, serological tests for syphilis, and tests for hepatitis B-associated antigen (HB$_s$Ag). After processing, the blood is then labeled appropriately; and the shipping documents for each container are completed and checked against the unit and master worksheet.

Exercises (262):

1. In accordance with what specific plan are blood donor centers designated?

2. Who is responsible for the control and technical supervision of the blood donor centers?

3. What is the minimum capability assigned to each donor center?

4. Blood donor centers are organized and operated in accordance with what regulations and manuals?

5. What should be checked before the donor leaves the physical examination area?

6. In order to prevent and reduce donor reaction, how long are the donors required to rest, under observation, in the recovery area?

7. How can we reap immeasurable benefits in the successful operation of the donor centers with reference to the treatment of the donors?

8. How are blood units which have been typed additionally for CDE routinely labeled?

263. Identify the function of ASWBPL in terms of its purpose and procedures in support of the Military Blood Program during peacetime and contingencies.

Armed Services Whole Blood Processing Laboratory (ASWBPL). The collected blood from donor centers is shipped via commercial or military air to the jointly staffed Armed Services Whole Blood Processing Laboratory, McGuire Air Force Base, New Jersey. After a thorough quality control inspection and double checking of group, Rh, and other characteristics, the blood is repacked at the ASWBPL and flown by commercial airlines to over 20 CONUS bases requesting blood.

In support of contingency/mobility operations, the request for blood is submitted to MBPO when the facility within that area is unable to obtain sufficient blood from within their own or neighboring resources. The MBPO will notify ASWBPL and will coordinate the response of the military blood program.

Blood is shipped to a central depository laboratory within the oversea command. There it is checked for adequate ice and overall condition, and trans-shipped to a central blood bank located in the immediate area of the conflict.

The Military Blood Program, in the Southeast Asia conflict, for the first time showed itself capable of carrying out a sustained mission based entirely on its own resources. This was made possible by a combination of well-motivated donors, improved laboratories, trained personnel, advanced technology, and dependable air transportation.

Exercises (263):

1. What is ASWBPL?

2. What is done with the blood shipped to ASWBPL from CONUS donor centers?

3. To what agency is the request for blood submitted when oversea bases are unable to obtain blood from within their own resources?

4. List some of the given factors responsible for successful accomplishment of the Military Blood Programs mission during the Southeast Asia conflict.

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ANSWERS FOR EXERCISES

CHAPTER I

Reference:
200 - 1. f.
200 - 2. l.
200 - 3. j.
200 - 4. d.
200 - 5. b.
200 - 6. g.
200 - 7. o.
200 - 8. e.
200 - 9. k.
200 - 10. h.
200 - 11. g.
200 - 12. m.
200 - 13. a.

201 - 1. c.
201 - 2. a.
201 - 3. d.
201 - 4. b.
201 - 5. g.
201 - 6. f.
201 - 7. e.
201 - 8. i.
201 - 9. j.

202 - 1. They could not.
202 - 2. It is never possible.
202 - 3. Determining the genotype.
202 - 4. The c antigen.
202 - 5. We phenotype using anti-c serum.
202 - 6. The c antigen.
202 - 7. She may be sensitized to antigens that are also possessed by the fetus if she should become pregnant.

203 - 1. The reactions between the very specific substances known as antigens and corresponding antibodies.
203 - 2. Protein, on occasion carbohydrates, rarely lipid.
203 - 3. A hapten, or carbohydrate portion coupled with a protein carrier.
203 - 4. The hapten.
203 - 5. Heteroimmunization.
203 - 6. Antigenic determinant.
203 - 7. Isoantigens.
203 - 8. Isoimmunization.
203 - 9. F. More rapid.
203 - 10. F. Rise higher and remain high for a longer period.
203 - 11. T.
203 - 12. T.

204 - 1. Antibodies are specific serum proteins produced in the lymphoid tissue as a resulting antigenic stimulation.
204 - 2. Gamma globulin.
204 - 3. Ultracentrifugation analysis, diffusion, immuno-electrophoresis, electrophoretic mobility.
204 - 4. It applies collectively to all protein molecules having antibody activity.
204 - 5. IgM, IgG, IgA, Ig D, and IgE.
204 - 6. Chemical, physical, and biological.
204 - 7. IgA.
204 - 8. The sedimentation constant.
204 - 9. IgG.
204 - 10. IgM.

205 - 1. Heavy chains.
205 - 2. Light chains.
205 - 3. 20,000 to 25,000.
205 - 4. Kappa (k) or lambda (λ).
205 - 5. The differences in the heavy chains.
205 - 6. Disulfide bonds.
205 - 7. The terminal region in which the amino acid sequence varies.
205 - 8. The sequence of amino acids in the variable region.

206 - 1. F. Primary response.
206 - 2. F. Macrophages.
206 - 3. F. Ribonucleic acid (RNA).
206 - 4. T.
206 - 5. F. Induction period.
206 - 6. T.
206 - 7. F. Memory.
206 - 8. F. Decreased from 10 to 1 days.
206 - 9. T.
206 - 10. F. Better.

207 - 1. l.
207 - 2. k.
207 - 3. l.
207 - 4. g.
207 - 5. m.
207 - 6. g.
207 - 7. h.
207 - 8. h.
207 - 9. j.
207 - 10. i.
207 - 11. i.
207 - 12. m.
207 - 13. g.
207 - 14. f.
207 - 15. e.
207 - 16. c.
207 - 17. d.
207 - 18. a.
207 - 19. n.

208 - 1. A thermolabile substance found in normal serum that is both necessary and responsible for hemolysis.
208 - 2. Anti-A, anti-B, anti-Le^a, anti-Le^b, anti-Jk^b, anti-K, anti-Fy^a.
280 - 3. Antibodies of the Rh system.
208 - 4. Cold *incomplete* anti-H.
208 - 5. Hemolysis occurs.

208 - 6. It denotes an antibody that adheres to the surface of red cells suspended in saline but fails to agglutinate them.
208 - 7. M, N, Fy^a alter complement-fixation properties.
208 - 8. The presence or absence of complement binding antibodies.
208 - 9. Heparin.
208 - 10. They bind Ca^{++} and Mg^{++} ions, both of which are required co-factors in the complement sequence.
208 - 11. IgA.
208 - 12. Anti-A, anti-B, anti-$PP_1P^k(T_ja)$, and anti-Le^a.

209 - 1. 37° C.
209 - 2. Anti-A, anti-Lewis, anti-M, and anti P_1.
209 - 3. Between 6.5 and 7.
209 - 4. 5.5.
209 - 5. The rate of antibody binding is greater initially; thus, the amount of time required to reach this equilibrium is shorter for some antibody-red cell reactions.

210 - 1. A measure of intensity of the electrical field due to ions in solution.
210 - 2. It provides a reaction medium in which the antibody binding to red cell antigens takes place.
210 - 3. Antibody bound to the cells will increase.
210 - 4. Autoagglutination and false positive results.
210 - 5. The electrostatic repulsion between one red cell and another.
210 - 6. The ionization of carboxyl groups of N-acetyl neuraminic acid residues (NANA) or the red cell membrane.
210 - 7. It decreases and facilitates the interaction of electropositive IgG with the negatively charged red cell.
210 - 8. Both the electronegative charge and zeta potential are reduced, thereby accelerating the second stage of agglutination.
210 - 9. Cause it to decrease, resulting in net agglutination.
210 - 10. Through the loss of sialic acid residues.
210 - 11. Causes an increase in the dielectric constant of the medium, increasing the charge dissipation characteristics.

211 - 1. Specific antibodies, which were transferred across the placenta from the mother.
211 - 2. The infant becomes jaundiced and the oxygen carrying capacity becomes inadequate.
211 - 3. D and c.
211 - 4. I_gG or γG 7-S.
211 - 5. They are not I_gG antibodies.

212 - 1. To remove the baby's coated cells and to replace them with other red cells that cannot react with passively acquired antibodies.
212 - 2. (a) Replaces destroyed erythrocytes, and (b) removes antibodies from the circulation.
212 - 3. Because the blood must be compatible with the antibodies in the mother's serum. These antibodies cross the placenta and coat the baby's cells. The coated cells are rapidly destroyed within the baby's circulatory system.
212 - 4. Preferably under 24 hours old and never more than 5 days old.
212 - 5. Use *low-titer* group O negative.
212 - 6. A titer of anti-A and anti-B less than 1:50.

213 - 1. It is given as an effective means of preventing maternal Rh alloimmunization.
213 - 2. Complete suppression of active immunity can be caused by administering a passive antibody.
213 - 3. A term used by modern investigators in place of *iso*. *Iso* refers to the intraspecies reaction.
213 - 4. Patients must be Rh negative and D^u negative, have no detectable anti-$Rh_o(D)$ antibody, and have an Rh positive or D^u positive newborn.
213 - 5. When the fetus or father is known to be Rh negative. Otherwise, Rh_oGAM is administered.
213 - 6. (a) Rh_o negative women whose serum contains anti-$Rh_o(D)$, (b) Rh_o negative women who deliver Rh negative babies, (c) Rh_o positive or D^u positive women.
213 - 7. Rh_oGAM should not be administered. The Rh_oGAM is specific for anti-Rh_o immunosuppression.
213 - 8. By a panel of known cell types.
213 - 9. (a) It can provide warning that a large number of Rh-positive red cells have entered the recipient's circulation; (b) it serves to prevent accidents due to mistakes in identification.
213 - 10. 72 hours.
213 - 11. The volume of Rh positive fetal cells which escapes into the maternal circulation is an unknown.
213 - 12. At normal blood bank temperature (4° to 6° C).

CHAPTER 2

214 - 1. "Naturally occurring" means that these antibodies occur without the stimulation of transfusion, injection, or pregnancy.
214 - 2. ABO antibodies are stimulated by substances in food, water, and air that have antigenic properties similar to those of A and B antigens.
214 - 3. Antibodies produced as a result of injections or pregnancies.
214 - 4. By natural exposure of body substances with antigenic properties identical, for all practical purposes, to those of the A and B antigens.
214 - 5. IgM.
214 - 6. IgG.
214 - 7. Anti-A and anti-B antibodies.
214 - 8. Ages 5 to 10 years.

215 - 1. H substance.
215 - 2. The O gene does not stimulate or change H substance; therefore, the amount of H substance in the cell does not diminish. In the case of A and B cells, when A or B antigen is produced, the H substance is mostly used up.
215 - 3. A mucopolysaccharide.
215 - 4. The cells of these group O individuals do not contain any H substance; hence, they can develop anti-H as well as anti-A and anti-B.
215 - 5. Body tissues and secretions.
215 - 6. The Se gene.
215 - 7. Secretors.
215 - 8. The brain and spinal cord.

216 - 1. T.
216 - 2. F. Anti-A_1.
216 - 3. F. Anti-A_1.
216 - 4. F. There may be a transfusion reaction.
216 - 5. T.
216 - 6. F. It will not react.
216 - 7. T.
216 - 8. T.
216 - 9. F. Absorption and elution studies.
216 - 10. F. Infrequent; have decreased amounts of B antigen.

217 - 1. Because tube testing is more reliable.
217 - 2. Contaminated and impotent antisera, cold agglutinins, other saline reactive agglutinins, and polyagglutinable erythrocytes.
217 - 3. Proof grouping, reverse grouping, and back grouping.
217 - 4. Serum grouping is a quality control procedure and, when performed, insures that we have correctly grouped the blood being tested.
217 - 5. Subgroups of A.
217 - 6. Serum grouping.
217 - 7. They must be prepared daily because red blood cells suspended in saline deteriorate rapidly.
217 - 8. To detect autoagglutination.

218 - 1. F. Nonreactive.
218 - 2. F. There are no agglutinogens.
218 - 3. T.
218 - 4. T.
218 - 5. F. Le(a+b-).

218 - 6. T.
218 - 7. F. Are agglutinated.
218 - 8. T.
218 - 9. T.
218 - 10. F. 25 percent.
218 - 11. F. Naturally occurring.

219 - 1. Because individuals who lack the substance in their saliva are Lewis negative and can become sensitized to the Lewis factor if transfused with Lewis-positive cells.
219 - 2. Reaction could be serious, or even a fatal hemolytic transfusion reaction could occur.
219 - 3. (a) Lewis antibodies are of the 19-S variety or IgM and thus do not pass through the placenta. (b) In the newborn infants, the Lewis substance is not yet adsorbed on the red cells.
219 - 4. Anti-Le^a + Le^b.
219 - 5. Blacks; because of the higher frequency of the phenotype Le (a–b–).
219 - 6. Le^a and Le^b reactivity of cells may be diminished by washing the cells.

220 - 1. F. Weiner.
220 - 2. T.
220 - 3. F. Hr′ = c and rh′ = C.
220 - 4. F. Not available.
220 - 5. F. CDE and D^u negative.
220 - 6. T.
220 - 7. F. Incubated for 1 hour.
220 - 8. T.
220 - 9. T.
220 - 10. F. More often in blacks than whites.

221 - 1. T.
221 - 2. F. hr^v.
221 - 3. F. C or D.
221 - 4. T.
221 - 5. F. Rh positives.
221 - 6. T.
221 - 7. F. A bar.
221 - 8. T.

222 - 1. e.
222 - 2. n.
222 - 3. h.
222 - 4. i.
222 - 5. j.
222 - 6. g.
222 - 7. d.
222 - 8. o.
222 - 9. c.
222 - 10. m.
222 - 11. l.
222 - 12. f.
222 - 13. l.
222 - 14. k.
222 - 15. b.

CHAPTER 3

223 - 1. Repelling electrical charges, and dimensions of the antibody molecule.
223 - 2. The antigen-antibody reaction taking place on the red cell remains invisible. To confirm the fact that antibody-antigen reaction had actually taken place on the cell surface, Coombs serum is added. The originally invisible antigen antibody reaction is thus made visible.
223 - 3. In the direct Coombs test, we are concerned with cells which have been coated with antibody *in vivo*, and in the indirect Coombs test, we are testing serum for Coombs-reactive antibodies or *in vitro* sensitization of red cells.
223 - 4. Rabbits, goats, and sheep.
223 - 5. Human gamma globulin.
223 - 6. Each animal's response to an injection of human gamma globulin is different. In order to get maximum reactivity and for standardization of the product, it must be blended.

224 - 1. T.
224 - 2. F. You may introduce extraneous human globulin.
224 - 3. F. Always run controls with every test.
224 - 4. F. Do not overcentrifuge.
224 - 5. T.
224 - 6. F. False-positive.
224 - 7. F. False-negative.
224 - 8. T.

225 - 1. d.
225 - 2. e.
225 - 3. d.
225 - 4. c.
225 - 5. f.
225 - 6. a, b.
225 - 7. h.

226 - 1. F. IgM and IgG classes.
226 - 2. T.
226 - 3. F. The important antigens.
226 - 4. T.
226 - 5. F. Macroscopically and microscopically.
226 - 6. T.
226 - 7. F. Add 1 drop of a 5 percent suspension of AHG-positive control cells to validate Coombs tests.
226 - 8. T.
226 - 9. F. Nonspecific cold-reacting antibody.
226 - 10. F. Controls of patient's cells and serum should be included.
226 - 11. T.
226 - 12. F. Not due.

227 - 1. Antigens.
227 - 2. Antibody screening.
227 - 3. The process of elimination.
227 - 4. Lewis, M, N, and P.
227 - 5. Patient's own red cells and serum.
227 - 6. Dosage is a condition in which cells from a person possessing a homozygous gene combination tend to react more strongly with specific antibodies than the cells of those persons with a heterozygous gene combination.
227 - 7. An antibody against a high frequency antigen may be present.
227 - 8. A cold antibody.
227 - 9. The patient may have a warm antibody.

228 - 1. (a) Presence of multiple antibodies; (b) a single antibody showing the property of dosage.
228 - 2. Tube 1. ~~D~~ C E ~~c~~ ~~c~~ K ~~k~~ Fy^a Fy^b.
Tube 2. C E ~~K~~ Fy^a ~~Fy^b~~.
Tube 5. C ~~E~~ ~~Fy^a~~.
Antibody identification: Anti-rh′(C).

229 - 1. To remove ionogenic surface groups and thus reduce surface charge.
229 - 2. Enzymes should be used only in those cases where there is a good probability of the presence of an enzyme reactive antibody.
229 - 3. In many instances, enzymes enhance the reaction of nonspecific agglutinins and prevent the reaction of antibodies in the MNS and Duffy systems by destroying their receptor sites.

230 - 1. Removing antibody from a serum by reacting it with the specific antigen, and then physically separating the antigen-antibody complexes from the serum.
230 - 2. a. Removal of cold or warm autoantibody to permit evaluation of coexisting alloantibody.

b. Removal of anti-A and/or anti-B from a serum which contains an unexpected antibody suitable for reagent used.
c. Separation of mixed antibodies in serum or eluate.
d. Showing that cells contain an antigen by showing their ability to remove antibody from serum.
230 - 3. One part undiluted serum to one volume of washed, packed cells.
230 - 4. 37° C.
230 - 5. Elution of antibody from the red cells may result.
230 - 6. Removal of an antibody that has been adsorbed onto red cells either *in vivo* or *in vitro*.
230 - 7. To demonstrate and identify antibodies on the red cells of umbilical cord blood or infant's blood in hemolytic disease of the newborn. Elution may be used to demonstrate and identify antibodies in a mixture.
230 - 8. 56° C; the same temperature at which the elution is performed.

231 - 1. T.
231 - 2. F. Highest.
231 - 3. F. It is not considered.
231 - 4. T.
231 - 5. T.
231 - 6. T.
231 - 7. F. A saline medium should be used.
231 - 8. F. Macroscopically.
231 - 9. F. Done at the same time.
231 - 10. F. These should be used consistently.

232 - 1. a. It makes the job easier and more orderly.
b. It protects the patient from simple administrative errors that could harm him.
c. It protects you, the technician, against charges of negligence in the event of transfusion problems.
232 - 2. a. SF 518, Medical Records—Blood or Blood Component Transfusion.
b. DD Form 572, Blood Donor Record.
c. Blood bank ledger.
232 - 3. Section I.
232 - 4. Section II.
232 - 5. Have the tubes properly labeled by the technician who drew the blood. DO NOT label the tubes yourself.
232 - 6. Section II.
232 - 7. How quickly the blood is needed.
232 - 8. Two copies; one copy.
232 - 9. To indicate that the number of units of blood or blood products requested have been successfully crossmatched and are available.

233 - 1. F. AFM 160-24.
233 - 2. F. Uniformity has become essential.
233 - 3. T.
233 - 4. F. Kept on all patients.
233 - 5. F. Five years.
233 - 6. T.

234 - 1. g.
234 - 2. a, g.
234 - 3. a.
234 - 4. a.
234 - 5. b, d.
234 - 6. b, d.
234 - 7. b.
234 - 8. b.
234 - 9. f.
234 - 10. f, h.
234 - 11. h.
234 - 12. f, h.
234 - 13. c.
234 - 14. d.
234 - 15. e.
234 - 16. j.
234 - 17. i.
234 - 18. i.

235 - 1. The prevention of a transfusion reaction and the assurance of maximum benefit of the transfusion to the patient.
235 - 2. Saline, albumin, and antihuman globulin serum.
235 - 3. Donor's cells and patient's serum.
235 - 4. A broad spectrum, donor antibody screening test.
235 - 5. Antiglobulin serum following incubation at 37° C for 15 to 30 minutes.
235 - 6. 22 to 30 percent albumin.
235 - 7. a. Antibodies present in the patient serum directed against antigens on the donor red cells.
b. Some errors in ABO grouping, labeling, and identification of donors and recipients.
235 - 8. None.

236 - 1. F. Human or nontechnical errors are more common.
236 - 2. T.
236 - 3. T.
236 - 4. F. Hemolysis and contamination are possible.
236 - 5. T.
236 - 6. F. 48 hours.
236 - 7. F. It is dangerous to be without blood for a patient if both need the unit at the same time.

237 - 1. F. He must be familiar with the procedures being utilized.
237 - 2. T.
237 - 3. F. It does not.
237 - 4. F. 70 percent plasma removed.
237 - 5. F. They must not be accepted.
237 - 6. T.

238 - 1. To give information as to temperatures and media where reactions occur, variability of reactions, and the percentage of incompatible donors.
238 - 2. Recheck ABO group, Rh types of incompatible donors and recipients. Recheck pilot sample number against donor units. Run an autocontrol with the antibody identification panel.
238 - 3. a. Error in ABO grouping of donor or recipient.
b. Error in identification of donor's or recipient's specimen.
c. Irregular antibodies or atypical antibodies, and technical errors.
238 - 4. The donor's or recipient's red cells may not contain the specific antigens that match the irregular antibodies in the serum of either recipient or donor.
238 - 5. Dirty glassware, bacterial contamination, chemical or other contaminants in reagents, fibrin clots, and overcentrifugation.

239 - 1. b, h, i.
239 - 2. b.
239 - 3. j.
239 - 4. i.
239 - 5. h.
239 - 6. h.
239 - 7. c.
239 - 8. a.
239 - 9. a.
239 - 10. d.
239 - 11. d.
239 - 12. g.
239 - 13. f.
239 - 14. e.

240 - 1. T.
240 - 2. F. Freezing and subsequent thawing will cause hemolysis.
240 - 3. T.
240 - 4. T.
240 - 5. F. Never.
240 - 6. F. It should not be returned to refrigeration for future use.

241 - 1. Any type.

241 - 2. The early signs and symptoms of hemolytic reactions are highly variable, and what appeared at first to be a very mild reaction may later turn out to be serious.

241 - 3. a. Improper identification of the recipient blood sample.
b. Infusion of blood into the wrong recipient.

241 - 4. Recheck for clerical errors.

241 - 5. a. Pre-transfusion blood of recipient.
b. Post-transfusion blood of recipient.
c. Pilot samples of donor blood.
d. Blood from donor bag implicated in the reaction.
e. Post-transfusion urine.

241 - 6. a. Post-transfusion serum compared with pre-transfusion.
b. Urine for red or *coke* color.

241 - 7. All the blood samples.

241 - 8. Pre- and post-transfusion samples.

241 - 9. Pre- and post-transfusion samples.

241 - 10. Bilirubin and urea nitrogen.

242 - 1. Virus causing *serum hepatitis* designated as Type B, or MS-2.

242 - 2. It is associated with spherical particles of 20 mm in diameter, tubular forms of similar width and the Dane particles.

242 - 3. Seriously high.

242 - 4. Type B.

243 - 1. f, d.
243 - 2. f.
243 - 3. a.
243 - 4. g.
243 - 5. b.
243 - 6. h.
243 - 7. c.
243 - 8. c, h.
243 - 9. e.
243 - 10. e.
243 - 11. d.
243 - 12. c.
243 - 13. c, g.
243 - 14. h.

244 - 1. Erroneous results can produce immediate, delayed, or even fatal results with *no* second chance available.

244 - 2. Kept up to date, evaluated periodically, and studied before changing a technique.

244 - 3. Microscopically or with some sort of magnification.

244 - 4. A chart graphically showing gradation of agglutination from weakly reactive up to 4+.

244 - 5. The typing and crossmatching should be double checked and countersigned by a person familiar and current with the detailed techniques and procedures being used.

244 - 6. (a) The telephone number of the medical equipment repair technician on call, (b) the location of alternate storage in case of equipment failure.

244 - 7. The programs evaluate the accuracy and methodology of the blood bank and compare proficiency in relation to other participating laboratories.

244 - 8. Use of weakly reactive antibodies and red cells of unusual phenotype.

245 - 1. F. Checked on the day of use.
245 - 2. F. Not deceleration.
245 - 3. T.
245 - 4. F. The RPM is a part of the G force calculation: $G = 0.00001118 \times RPM^2 \times R(\text{radius})$.
245 - 5. T.
245 - 6. F. The timer should be checked with a stop watch and correction marked on the face of the timer.
245 - 7. F. Longer time and greater force.
245 - 8. F. Each centrifuge should be calibrated.
245 - 9. T.
245 - 10. F. Time.
245 - 11. T.
245 - 12. F. 5 minutes.
245 - 13. T.
245 - 14. F. 45° to 50° C.
245 - 15. F. All new thermometers should be checked.

246 - 1. Quality, dependability.
246 - 2. Bureau of Biologics of the FDA.
246 - 3. Laboratory.
246 - 4. Reactivity.
246 - 5. Specificity, potency, avidity.
246 - 6. Avidity.
246 - 7. Reactivity.
246 - 8. Specificity.
246 - 9. Discarded.
246 - 10. Weak Rh_o (D).
246 - 11. Hemolysis or darkening of red cells, crenation of RBCs, and Rouleaux forming properties.

CHAPTER 4

247 - 1. F. DD Form 572.
247 - 2. F. Useful in protecting the donor and the patient or recipient.
247 - 3. T.
247 - 4. T.
247 - 5. F. It is the responsibility of the blood bank technician to notify Environmental Health Services.
247 - 6. F. DD Form 572.
247 - 7. F. Bears the accession number which helps to identify it.

248 - 1. AFM 160-24, *Standards for Blood Banks and Transfusion Services*, and AFM 160-50, *Technical Methods and Procedures of the American Association of Blood Banks*.

248 - 2. To determine whether giving blood will in any way harm the donor and to determine whether transfusion of the unit will in any way harm the recipient.

248 - 3. 110 pounds (50 kg).

248 - 4. Between 50 and 100 beats per minute.

248 - 5. The systolic blood pressure shall be between 90 and 180 mm of mercury and the diastolic should not exceed 100 mm.

248 - 6. None. They should be deferred.

248 - 7. Defer him temporarily.

248 - 8. Yes. The hemoglobin shall be no less than 12.5 gm per 100 ml for female donors.

249 - 1. F. They are accepted if less than 21 years of age and are married.
249 - 2. F. 8 weeks.
249 - 3. T.
249 - 4. T.
249 - 5. F. Cause for refection.
249 - 6. F. May be accepted provided they have not taken prophylactic medication in 48 hours.
249 - 7. T.
249 - 8. F. For at least 6 months.
249 - 9. T.
249 - 10. F. Aspirin or aspirin containing compounds depress platelet function for 12 to 18 hours.
249 - 11. T.
249 - 12. T.
249 - 13. F. It is a cause for rejection.
249 - 14. T.
249 - 15. F. Immigrants or visitors from endemic areas may be accepted as blood donors 3 years after departure from the area if they have been found asymptomatic in the interim.
249 - 16. T.

250 - 1. A donor with a history of viral hepatitis is permanently excluded.
250 - 2. He is permanently excluded.
250 - 3. Such a donor is permanently excluded.
250 - 4. In case hepatitis develops.
250 - 5. They will not donate blood IAW AFR 160-26, *Air Force Blood Program*.

251 - 1. Licensed physician.
251 - 2. Container, donor set.
251 - 3. Citrate phosphate dextrose.
251 - 4. Centrifugation, gravity.
251 - 5. Transfer pack.
251 - 6. Thrombocytes, cells.
251 - 7. At once.
251 - 8. Valve.
251 - 9. 30, moisture.

252 - 1. Plastic spring scale, blood collection balance, and a Hemolator.
252 - 2. The weight goes up when the bag is filled and the bag goes down causing a pinch in the tube, thus restricting the blood flow.
252 - 3. The Hemolator automatically agitates the bag, mixing the blood and the anticoagulant during collection. Vacuum used with the device causes negative pressure to the exterior of the blood bag unit. When the quantity desired is collected, the flow of blood automatically stops.
252 - 4. Check the Blood Donor Record to be sure that the donor is eligible to give blood.
252 - 5. This elevates the person's head and may contribute to vertigo.
252 - 6. These questions are psychologically encouraging the donor to react.
252 - 7. a. Be certain that a physician in the building can be reached.
  b. At least another person is within calling distance so that you can summon aid if the donor develops a reaction.

253 - 1. F. Surgical soap.
253 - 2. F. Move from the intended venipuncture site to the periphery.
253 - 3. T.
253 - 4. T.
253 - 5. F. Poor technique. This rinses bacteria from the hands into the stock container.
253 - 6. T.
253 - 7. F. Remove the needle and protect the donor from biting his tongue by using a tongue depressor. Consult the physician in attendance.

254 - 1. T.
254 - 2. F. Loose knot.
254 - 3. F. Low enough to allow gravity collection.
254 - 4. F. Do venipuncture immediately.
254 - 5. F. The flow is stopped manually.
254 - 6. T.
254 - 7. T.
254 - 8. F. Strip donor tubing as completely as possible into the bag, working quickly before initiation of coagulation occurs.
254 - 9. T.
254 - 10. F. Refrigerate as soon as possible.

255 - 1. The cell membrane loses lipid.
255 - 2. Potassium leaks from the red cells, sodium enters, and equilibrium between the two ions is approached.
255 - 3. The falling pH leads to inhibit enzymes necessary for the phosphorylation of glucose, the first step in glycolysis.
255 - 4. Adenosine triphosphate (ATP).
255 - 5. There is an increased affinity of the red cell hemoglobin for oxygen.
255 - 6. The capacity to deliver oxygen to the recipient's tissues in turn is lowered.
255 - 7. They allow the red cell to maintain concentrations of adenosine triphosphate to assure adequate viability for 21 days' storage at 4° C.
255 - 8. By the requirement that at least 70 percent of the transfused red cells must remain in the circulation 24 hours after transfusion.
255 - 9. The two millimolar (mM) phosphate present in CPD contributes to the adenosine phosphate pool.
255 - 10. Because of the higher pH of CPD-stored blood.
255 - 11. Cardiopulmonary-bypass surgery and exchange transfusions.
255 - 12. 48 hours.

256 - 1. Blood, blood components, reagents, blood samples.
256 - 2. 1, 6, 2.
256 - 3. Temperature recording, alarm system.
256 - 4. Weekly, refrigerator.
256 - 5. Permanent.
256 - 6. Outdated.

257 - 1. An appropriate amount of a cryoprotective agent, such as glycerol solution, is added to red cells and stored at ultra-low temperatures for long periods of time. Prior to transfusion, the cells are thawed and the glycerol is removed by washing.
257 - 2. Freezing arrests the metabolism.
257 - 3. The removal of plasma and the great majority of the nonerythrocytic formed elements.
257 - 4. Platelet concentrates, fresh frozen plasma, and cryoprecipitates.
257 - 5. a. The clinical problems associated with post-transfusion hepatitis.
  b. Transfusion of red cell antibodies.
  c. Tissue antigens present on white cells and platelets.
  d. Plasma protein antigens and antibodies.
  e. Leukocyte-borne viral disease.
  f. Hyperkalemia and anticoagulant toxicity.
257 - 6. Infectivity of hepatitis and HL-A antigenicity are reduced.
257 - 7. Extensive washing removes the plasma containing IgA.
257 - 8. Elimination of plasma antibodies and microemboli in donor blood.

258 1 The high-glycerol, slow freezing method, and the low-glycerol, rapid-freezing method.
258 - 2. a. High-glycerol, slow freezing; storage at -80° or -85° C.
  b. Low-glycerol, rapid-freezing; storage at -150° or -196° C.
258 - 3. He took advantage of the fact that red cells in a low-salt, low-pH medium will agglomerate into large masses.
258 - 4. It leads to rapid sedimentation and thus eliminates the need for centrifugation in washing and deglycerolizing the red cells.
258 - 5. The removal of plasma and for the final red cell concentration.
258 - 6. EDTA.
258 - 7. By adding 500 ml of 50 percent glucose to the red cells.
258 - 8. 75 to 85 percent.
258 - 9. A solution of glycerol, sodium chloride, and mannitol.
258 - 10. Liquid nitrogen at -196° C, or gaseous phase at -150° C.
258 - 11. Ultra-low temperature systems must be maintained by liquid nitrogen which may be expensive in some regions, and storage and transportation vessels are also cumbersome and expensive.

259 - 1. F. 1° C and 10° C.
259 - 2. F. Air.
259 - 3. F. Wet ice.
259 - 4. T.
259 - 5. F. AFR 168-3.
259 - 6. F. DD Form 573, Shipping Inventory of Blood Collection.
259 - 7. F. Release the unit or crossmatch another unit.
259 - 8. F. DD Form 573.
259 - 9. T.
259 - 10. F. The transportation officer.
259 - 11. T.
259 - 12. T.
259 - 13. T.

260 - 1. T.

260 - 2. F. All emergencies ranging from local disasters to general war.
260 - 3. T.
260 - 4. F. Military Blood Program Office.
260 - 5. T.
260 - 6. F. AFR 160-26, *Air Force Blood Program*.
260 - 7. F. They donate without payment.
260 - 8. F. Only those already licensed by the FDA.
260 - 9. F. Will not be used; exceptions made by the hospital commander in emergency or unusual geographical situations.
260 - 10. F. It is a command responsibility.
260 - 11. T.

261 - 1. To provide the Nation's requirement for whole blood, blood derivatives, and plasma in the event of mobilization or national emergency.
261 - 2. The Office of Emergency Planning.
261 - 3. The Department of Defense.

262 - 1. Volume I of the USAF War and Mobilization Plan.
262 - 2. The director of base medical services.
262 - 3. They must be capable of collecting, classifying, storing, and shipping 600 units of whole blood each 30 days.
262 - 4. AFM 160-24, AFM 160-50, and AFR 168-3.
262 - 5. His DD Form 572.
262 - 6. 10 to 15 minutes.
262 - 7. By treating the donors as individuals and showing them that we are concerned for their safety and welfare.
262 - 8. Routine testing for additional blood types is not recommended.

263 - 1. The jointly staffed Armed Services Whole Blood Processing Laboratory.
263 - 2. Quality control inspection, double check on group, Rh, and repacking and shipment via commercial airlines to bases requesting blood.
263 - 3. The Military Blood Program Office (MBPO).
263 - 4. a. Well-motivated donors.
b. Improved laboratories.
c. Trained personnel.
d. Advanced technology.
e. Dependable air transportation.

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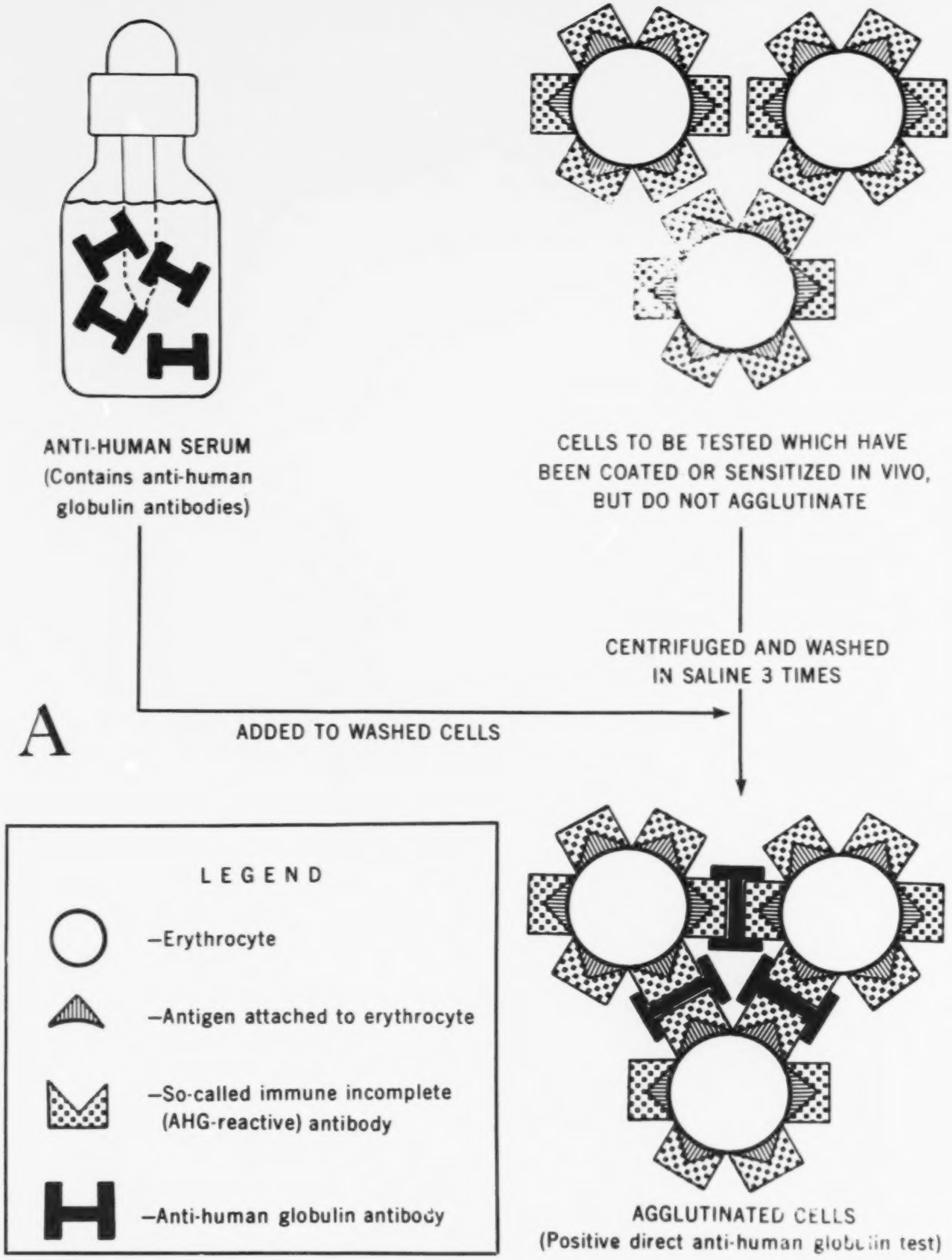
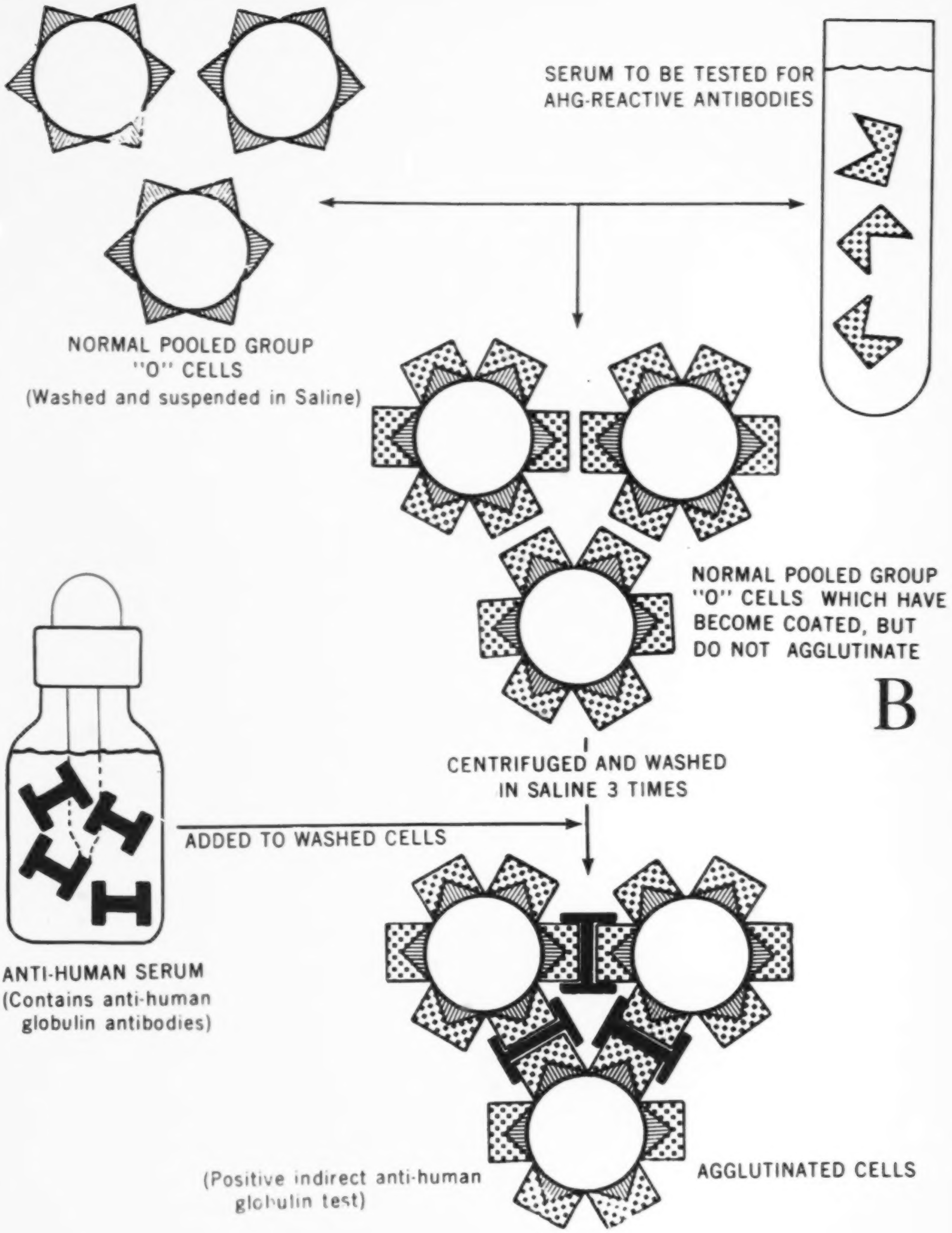

ANTI-HUMAN SERUM
(Contains anti-human globulin antibodies)
CELLS TO BE TESTED WHICH HAVE BEEN COATED OR SENSITIZED IN VIVO, BUT DO NOT AGGLUTINATE
CENTRIFUGED AND WASHED IN SALINE 3 TIMES
A
ADDED TO WASHED CELLS
LEGEND
—Erythrocyte
—Antigen attached to erythrocyte
—So-called immune incomplete (AHG-reactive) antibody
—Anti-human globulin antibody
AGGLUTINATED CELLS
(Positive direct anti-human globulin test)


SERUM TO BE TESTED FOR
AHG-REACTIVE ANTIBODIES
NORMAL POOLED GROUP
"O" CELLS
(Washed and suspended in Saline)
NORMAL POOLED GROUP
"O" CELLS WHICH HAVE
BECOME COATED, BUT
DO NOT AGGLUTINATE
B
CENTRIFUGED AND WASHED
IN SALINE 3 TIMES
ADDED TO WASHED CELLS
ANTI-HUMAN SERUM
(Contains anti-human
globulin antibodies)
(Positive indirect anti-human
globulin test)
AGGLUTINATED CELLS

Foldout 1. Direct Coombs test (A) and indirect Coombs test (B).

SHIPPING INVENTORY OF BLOOD COLLECTIONS

TO BE COMPLETED BY COLLECTING CENTER

ACTIVITY NUMBER

COLLECTING CENTER AND ADDRESS *(Include ZIP Code)*

NAME AND ADDRESS OF CONSIGNEE *(Include ZIP Code)*

SHIPPED

DATE

HOUR

CARRIER SHIPPED BY

TRAIN OR FLIGHT NUMBER

HOUR OF DEPARTURE

SHIPPING CONTAINER NO.

BLOOD NUMBER	DATE COLLECTED	BLOOD GROUP	RH	SEROLOGY	BLOOD NUMBER	DATE COLLECTED	BLOOD GROUP	RH	SEROLOGY
1					13				
2					14				
3					15				
4					16				
5					17				
6					18				
7					19				
8					20				
9					21				
10					22				
11					23				
12					24				

REMARKS

TYPED NAME OF SHIPPING OFFICER

SIGNATURE

TO BE COMPLETED BY RECEIVER

RECEIVED AT

DATE

HOUR

REMARKS *(Note discrepancies opposite blood numbers)*

TYPED NAME OF RECEIVING OFFICER

SIGNATURE

DISTRIBUTION OF COPIES: *One copy retained by Receiver, original returned to Collecting Center (Forwarded by Receiver), one copy retained by Collecting Center.*

DD FORM 1 MAR 69 573

REPLACES EDITION OF 1 MAR 66, WHICH WILL BE USED.

GPO 865-600

DONOR NUMBER	DATE COLLECTED	DATE EXPIRES	INTEGRAL TUBE NUMBER (Optional)	BLOOD GROUP AND RH
BAG ISSUE ☐ SINGLE ☐ DOU ☐ TRIP OTHER	ANTICOAGULANT ☐ CPD ☐ ACD ☐ OTHER	COLLECTION STARTED (Time)	COLLECTION ENDED (Time)	

BLOOD PRODUCTS PREPARED (Circle if Required)

WHOLE BLOOD (Human)
RED BLOOD CELLS (Human)
WHOLE BLOOD (Human) MODIFIED
SINGLE DONOR PLASMA (Human) FRESH FROZEN
SINGLE DONOR PLASMA (Human) FRESH FROZEN PLATELETS REMOVED
PLATELET CONCENTRATE (Human)
LEUCOCYTES (Human)
CRYOPRECIPITATED ANTIHEMOPHILIC FACTOR (Human)
OTHER ____________

____
Signature of Phlebotomist

REMARKS

1. DONOR'S NAME (Last, First, Middle Initial)	2. GRADE	3. SSN	7. FOR OFFICE USE
4. AGE	5. SEX	6. ORGANIZATION/STATION OR ADDRESS AND PHONE	

MEDICAL QUESTIONNAIRE	YES	NO	MEDICAL QUESTIONNAIRE	YES	NO	MEDICAL QUESTIONNAIRE	YES	NO
8 Have you donated blood in the past 9 weeks?			21. Have you had smallpox vaccination or other imm. in the past 2 weeks?			34. Have you had a tattoo? Ears pierced/acupuncture in the past 6 months?		
9 Have you ever been rejected as a blood donor?			22. Have you been vaccinated for German Measles in the past 2 months?			35. Have you ever injected any drugs into your veins or skin?		
10 Do you have any illness now?			23. Have you ever had convulsions or fainting spells? donor reaction?			36. Have you ever had venereal disease? ever been treated for syphilis?		
11 Do you have a cold, flu, Grippe, or sore throat (URI)?			24. Have you been immunized to human cells/red blood cell antigens?			37. Have you had a recent unexplained weight loss of over 10 pounds?		
12 Have you ever coughed up or vomited blood?			25. Have you received blood or plasma in the past 6 months?			38. Do you have skin infections? Frequent boils?		
13 Are you under a doctor's care now for any reason?			26. Have you donated plasma in the past 48 hours? (Plasmapheresis)?			39. Do you have any illnesses which are not covered by the questionnaire?		
14 Have you had tuberculosis? Kidney disease? Liver disease?			27. Have you had malaria? Taken antimalarial drugs in the past 3 years?			40. (Women) are you pregnant now or during the past 6 weeks?		
15 Have you experienced chest pain or shortness of breath?			28. Have you been outside the U.S. in the past 3 years?			41. Are you in good health generally/do you feel well now?		
16 Have you had heart trouble, rheumatic heart disease? Asthma?			29. Have you had surgery or serious illness in the past 6 months?					
17 Have you had lung or stomach disease? Cancer, diabetes, ulcers?			30. Have you ever had hepatitis (yellow jaundice)?					
18 Have you had lymph gland disease, leukemia, or infectious mono?			31. Have you been exposed to hepatitis in the past 6 months?					
19 Have you had tooth extraction surgery in the past 3 days?			32. Have you taken any drugs? Medications, intoxicants in the past 48 hours?					
20 Have you had rabies vaccination in the past year?			33. Have you taken any habit forming/hallucinogenic drug within 48 hrs.?					

DONOR HISTORY REMARKS

42. WEIGHT	43. TEMPERATURE	44. PULSE	45. BLOOD PRESSURE	46. HGB HCT

47. ARMS INSPECTION SATISFACTORY ☐ YES ☐ NO	48. GENERAL APPEARANCE ☐ SAT ☐ UNSAT	49. DONOR REJECTED ☐ NO ☐ YES ☐ PERM ☐ TEMP	50. AUTOLOGOUS DONOR ☐ YES ☐ NO	51. SUITABLE FOR TRANSFUSION TO OTHER PATIENTS ☐ YES ☐ NO ☐ N/A

Answers 1 through 50 are correct to the best of my knowledge and belief and I understand that my answers are important in determining my individual fitness to donate blood. I am voluntarily donating approximately 450 ml of blood to the Armed Forces for use in any way they may deem advisable. I understand that I should not engage in strenuous exercise or hazardous activity on the day of donation.

____________ ____________
Signature of Donor Date

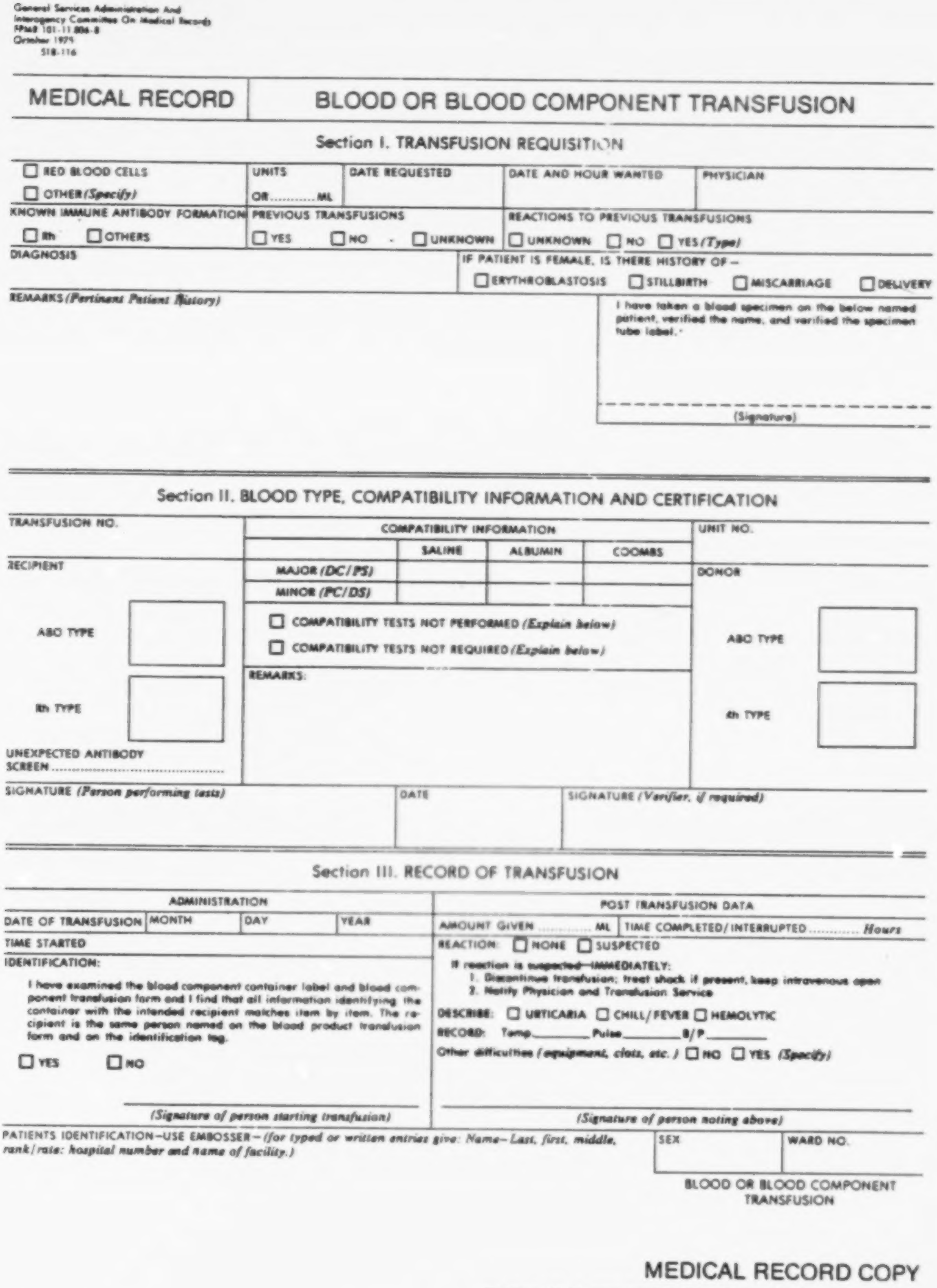
SIGNATURE OF INDIVIDUAL RESPONSIBLE FOR DETERMINING DONOR ELIGIBILITY

SIGNATURE OF MEDICAL REVIEWER

DD Form 572 EDITION OF 1 JAN 71 IS OBSOLETE

PROPOSED

DEPARTMENT OF DEFENSE MILITARY BLOOD PROGRAM
BLOOD DONOR RECORD



General Services Administration And
Interagency Committee On Medical Records
FPMR 101-11.806-8
October 1975
518-116

MEDICAL RECORD | **BLOOD OR BLOOD COMPONENT TRANSFUSION**

Section I. TRANSFUSION REQUISITION

☐ RED BLOOD CELLS
☐ OTHER *(Specify)*
UNITS
OR .... ML
DATE REQUESTED
DATE AND HOUR WANTED
PHYSICIAN

KNOWN IMMUNE ANTIBODY FORMATION ☐ Rh ☐ OTHERS
PREVIOUS TRANSFUSIONS ☐ YES ☐ NO ☐ UNKNOWN
REACTIONS TO PREVIOUS TRANSFUSIONS ☐ UNKNOWN ☐ NO ☐ YES *(Type)*

DIAGNOSIS
IF PATIENT IS FEMALE, IS THERE HISTORY OF – ☐ ERYTHROBLASTOSIS ☐ STILLBIRTH ☐ MISCARRIAGE ☐ DELIVERY

REMARKS *(Pertinent Patient History)*

I have taken a blood specimen on the below named patient, verified the name, and verified the specimen tube label.

(Signature)

Section II. BLOOD TYPE, COMPATIBILITY INFORMATION AND CERTIFICATION

TRANSFUSION NO.

UNIT NO.

COMPATIBILITY INFORMATION

	SALINE	ALBUMIN	COOMBS
MAJOR *(DC/PS)*			
MINOR *(PC/DS)*			

☐ COMPATIBILITY TESTS NOT PERFORMED *(Explain below)*
☐ COMPATIBILITY TESTS NOT REQUIRED *(Explain below)*

REMARKS:

RECIPIENT
ABO TYPE
Rh TYPE
UNEXPECTED ANTIBODY SCREEN ....

DONOR
ABO TYPE
Rh TYPE

SIGNATURE *(Person performing tests)*
DATE
SIGNATURE *(Verifier, if required)*

Section III. RECORD OF TRANSFUSION

ADMINISTRATION

DATE OF TRANSFUSION MONTH DAY YEAR
TIME STARTED
IDENTIFICATION:

I have examined the blood component container label and blood component transfusion form and I find that all information identifying the container with the intended recipient matches item by item. The recipient is the same person named on the blood product transfusion form and on the identification tag.

☐ YES ☐ NO

(Signature of person starting transfusion)

POST TRANSFUSION DATA

AMOUNT GIVEN .... ML
TIME COMPLETED/INTERRUPTED .... *Hours*
REACTION: ☐ NONE ☐ SUSPECTED

If reaction is suspected—IMMEDIATELY:
1. Discontinue transfusion; treat shock if present, keep intravenous open
2. Notify Physician and Transfusion Service

DESCRIBE: ☐ URTICARIA ☐ CHILL/FEVER ☐ HEMOLYTIC
RECORD: Temp.________ Pulse________ B/P________
Other difficulties *(equipment, clots, etc.)* ☐ NO ☐ YES *(Specify)*

(Signature of person noting above)

PATIENTS IDENTIFICATION—USE EMBOSSER—*(for typed or written entries give: Name—Last, first, middle, rank/rate: hospital number and name of facility.)*
SEX
WARD NO.

BLOOD OR BLOOD COMPONENT
TRANSFUSION

MEDICAL RECORD COPY

Foldout 2. Forms used in blood transfusion service. New

STOP- **1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.** **2. USE NUMBER 2 PENCIL ONLY.**

90413 02 23

EXTENSION COURSE INSTITUTE
VOLUME REVIEW EXERCISE

LABORATORY PROCEDURES IN BLOOD BANKING AND IMMUNOHEMATOLOGY

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that item numbers on answer sheet are sequential in each column.
3. Use a medium sharp #2 black lead pencil for marking answer sheet.
4. Write the correct answer in the margin at the left of the item. (When you review for the course examination, you can cover your answers with a strip of paper and then check your review answers against your original choices.) After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if a all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DONT'S:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than a #2 black lead pencil.

NOTE: NUMBERED LEARNING OBJECTIVE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Learning Objective Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Learning Objectives* indicated by these *Numbers.* The VRE results will be sent to you on a postcard which will list the *actual* VRE *items you missed.* Go to the VRE booklet and locate the *Learning Objective Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (200) Which of the following may be identified by routine grouping and typing?

   a. Phenotype.
   b. Genotype.
   c. X chromosome.
   d. Sex-linked characteristics.

2. (200) What is the number of genes required to produce hemophilia in males?

   a. One.
   b. Two.
   c. Twenty-three.
   d. Forty-six.

3. (201) What are the possible gene combinations if one parent is AO and the other is AB?

   a. AA, AO only.
   b. BB, OO only.
   c. OO, BO only.
   d. AA, AO, AB, BO.

4. (201) Which of the following terms refers to a particular position of genes on a chromosome?

   a. Heterozygous.
   b. Homozygous.
   c. Loci.
   d. Alleles.

5. (202) Next to D and D^u in the Rh series, which antigen is considered most antigenic?

   a. c.
   b. e.
   c. E.
   d. C.

6. (203) What part of the antigen determines the specificity?

   a. The protein carrier.
   b. Isoantigen.
   c. The hapten.
   d. The lipid portion.

7. (204) All of the following methods of detection and analysis have indicated the existence of many different forms of antibody globulin except which of the following?

   a. Diffusion,
   b. Precipitation.
   c. Immunoelectrophoresis.
   d. Ultracentrifugation analysis.

8. (204) The sedimentation constant method for classifying antibodies according to their molecular weights is expressed in

   a. Svedberg units.
   b. reaction units.
   c. angstroms.
   d. moles.

9. (204) Antibodies of the Le, MNS, and P systems are macroglobulins associated with which immunoglobulin?

   a. IgA.
   b. IgM.
   c. IgG.
   d. IgD.

10. (205) What determines the exact specificity of the antibody in terms of the basic immunoglobulin structure?

    a. Sulfide bonds.
    b. The terminal region.
    c. The difference in the heavy chains.
    d. The sequence of amino acids in the variable region.

11. (206) During the sequence of events reguired for antibody formations there is a lag of 7 to 10 days in time which is called the

a. reduction period.
b. lag phase.
c. induction period.
d. amamnestic response period.

12. (207) Which of the following is a type of false agglutination?

a. Nonspecific clumping.
b. Rouleaux.
c. Prozone.
d. Postzone.

13. (207) Autoagglutination occurs most frequently at what temperature?

a. 56° C.
b. 37° C.
c. 20° C.
d. 5° C.

14. (207) Which of the following types of agglutination is most likely to occur in certain patients with sepsis and does not occur as a rule when fresh, sterile sera are used?

a. Polyagglutination.
b. Autoagglutination.
c. Pseudoagglutination.
d. Panagglutination.

15. (207) In antigen-antibody reactions, an excess of antigen may result in

a. hemolysis.
b. a weak reaction.
c. too much reaction.
d. autoagglutination.

16. (208) The thermolabile substance responsible for hemolysis of red blood cells in antigen-antibody reaction is

a. albumin.
b. complement.
c. macroglobulin.
d. gamma globulin.

17. (208) What term denotes an antibody that adheres to the surface of red cells suspended in saline, but fails to agglutinate them?

a. Complete antibody.
b. Complement binding.
c. Incomplete antibody
d. Hemolytic antibody.

18. (209) Incubation times for routine laboratory procedures may be relatively short because the

a. rate of antibody binding is delayed.
b. rate of antibody binding is greatest at initial incubation.
c. optimum temperature and pH are reached rapidly.
d. optimum mixture of antigen-antibody is present.

19. (210) The electrostatic repulsion between one red cell and another is termed

a. ionic strength.
b. ionic potential.
c. zeta potential.
d. zeta ionization.

20. (211) What two Rh antigens are most commonly responsible for isoimmunization in pregnancy?

a. Anti-D and anti-c.
b. Anti-D and anti-e.
c. Anti-E and anti-e.
d. Anti-C and anti-c.

21. (212) If the mother is group A and the infant is group B, which type of "low titer" blood is preferable for exchange transfusions?

a. A negative.
b. B negative.
c. AB negative.
d. O negative.

22. (212) What are two primary benefits of an exchange transfusion?

a. Replaces destroyed erythrocytes and removes antibodies.
b. Replaces destroyed leukocytes and removes antibodies.
c. Restores oxygen to tissues and removes antibodies.
d. Restores oxygen to tissues and replaces destroyed erythrocytes.

23. (213) The use of Rh_o immune globulin solution is based upon the principle that complete suppression of active immunity can be caused by administering what factor?

a. A passive antibody.
b. A passive antigen.
c. An active antibody.
d. An active antigen.

24. (214) What type of immunoglobulin antibodies are natually occurring antibodies?

a. IgA.
b. IgD.
c. IgG.
d. IgM.

25. (215) What is the precursor substance that, under genetic stimulation, produces A or B antigens?

a. O-determinant.
b. H-substance.
c. O-gene.
d. Anti-H antibody.

26. (215) What gene controls the secretion of A and B substances into the body fluids?

a. Se.
b. Dominant.
c. Recessive.
d. H.

27. (216) The presence of weak A antigen can be confirmed by what two tests?

a. Adsorption and dilution.
b. Adsorption and elution.
c. Absorption and elution.
d. Absorption and dilution.

28. (217) Which of the following is not a source of error in ABO grouping?

a. Contaminated and impotent antisera.
b. Polyagglutinable erythrocytes.
c. Cold agglutinins.
d. Rh antibodies coating the cells.

29. (218) Sensitization due to a Lewis antigen is rarely seen because normally these antibodies will not react

a. at low temperatures.
b. at body temperatures.
c. with complement.
d. with A or B cells.

30. (218) Lewis antibodies, because they are found in individuals who have received no known antigenic stimulation, are considered as what type of antibodies?

a. Immune.
b. Atypical only.
c. Naturally occurring only.
d. Both atypical and naturally occurring.

31. (219) Why should Lewis typing, Le^a and Le^b, always be done with controls?

a. Reactivity may be enhanced by washing the cells.
b. Reactivity may be diminished by washing the cells.
c. Reactivity may be enhanced by saline controls.
d. Reactivity may be diminished by saline controls.

32. (220) D^u positive blood is commonly detected by using which of the following procedures?

a. Antibody elution.
b. Complete genotyping.
c. Direct Coombs.
d. Indirect Coombs.

33. (221) The LW antigen is more strongly expressed on which of the following types of cells?

a. Rh-positive.
b. Rh-negative.
c. D^u positive.
d. CE positive.

34. (222) When the reaction is enhanced by cold temperatures and the strength varies considerably on different positive persons, the antigen most likely suspected is which of the following?

a. K.
b. Fy^a.
c. P_1.
d. Jk^a.

35. (223) What antigen is used to stimulate animals in the production of the antibody for Coombs serums?

a. Bovine albumin.
b. Bovine gamma globulin.
c. Rabbit gamma globulin.
d. Human gamma globulin.

36. (224) Cells must be thoroughly washed before Coombs testing to remove

a. globulin.
b. complement.
c. albumin.
d. antibodies.

37. (224) Which of the following conditions may produce a false-negative Coombs test?

a. Incubation temperature above the optimum.
b. Scratches in the glassware.
c. The presence of multivalent cations.
d. Bacterial contamination of cells or serum.

38. (225) Which of the following is a hypertensive drug which may induce an antibody serologically identical to the IgG autoantibody or warm autoimmune hemolytic anemia?

a. Cephalothin.
b. Alpha-methyldopa (Aldomet).
c. Chloropromide.
d. Quinadine.

39. (225) The process in which an antibody formed by a drug combines with the circulating drug and the antibody complex then attaches to the red cells is called

a. drug induced adsorption.
b. autoimmune adsorption.
c. immune complex adsorption.
d. autoimmune response.

40. (226) Immune antibodies and blood groups are usually of which of the following classes?

a. IgA and IgE.
b. IgA and IgD.
c. IgD and IgE.
d. IgM and IgG.

41. (227) Stronger than normal reactions that occur with specific combinations of genes are known as

a. genotype.
b. phenotype.
c. dosage.
d. heterozygous.

42. (228) What does different strengths of reaction or reactions in different phases of testing in the cell panel indicate?

a. Multiple antibodies and dosage.
b. Single antibody present and high potency Coombs reagent.
c. Multiple antigens and high potency Coombs serum.
d. Single antibody and low potency Coombs serum.

43. (229) How might the use of enzymes prevent the reactions of antibodies such as Duffy, M, N, and S?

a. By neutralizing the ionic concentration of the medium.
b. By destroying the receptor sites.
c. By reducing the ionic concentration of the medium.
d. By destroying the ionic charge of the medium.

44. (230) What is the usual volume between undiluted serum and washed packed cells when performing the absorption procedure?

a. 1:1.
b. 2:1.
c. 1:2.
d. 1:3.

45. (230) The technique useful in removing unwanted antibodies that have been adsorbed on the red cells either in vivo or in vitro is called

a. absorption.
b. neutralization.
c. dilution.
d. elution.

46. (231) In the antibody titer technique, if the antibody is diluted with saline, what type of medium should be used for the cell suspension?

a. saline.
b. albumin.
c. enzyme.
d. a one-to-one ratio of saline and albumin.

47. (232) After a transfusion is completed the suspense copy of the SF 518 should be

a. filed.
b. retained.
c. destroyed.
d. returned to the transfusion service.

48. (233) Which of the following Air Force manuals establishes and maintains uniform blood banking standards for military departments that will be compatible with those of the civilian field of blood banking?

a. AFM 160-24.
b. AFM 160-47.
c. AFM 160-50.
d. AFM 168-4.

49. (234) Which of the following components may be stored for autologous transfusion, or for stockpiling of rare red cell types?

a. Leukocyte poor red blood cells.
b. Whole blood (cryoprecipitate and/or platelets removed).
c. Red blood cells.
d. Frozen red blood cells.

50. (234) Leukocytes are removed by differential sedimentation or centrifugation in the preparation of which of the following components?

a. Whole blood (cryoprecipitate and/or platelets removed).
b. Single donor plasma (cryoprecipitate removed).
c. Leukocyte poor red blood cells.
d. Frozen red blood cells.

51. (235) Which of the following statements best describes the primary purpose of the crossmatch?

a. To select healthy donors.
b. To identify unknown antigens.
c. To identify unknown antibodies.
d. To prevent a transfusion reaction.

52. (235) Which of the following tests is often performed in place of a minor crossmatch?

a. Enzyme.
b. Donor antibody screening.
c. Cell panel.
d. Coombs.

53. (235) The crossmatch procedure will do all of the following except detect

a. antibodies present in the recipient serum directed against antigens on the donors red cells.
b. some errors in ABO grouping.
c. some errors in labeling and identification of donors and recipients.
d. all recipient antibodies, thus giving assurance that no isoimmunization will occur.

54. (236) In accordance with AFM 168-4, how long should blood earmarked for a patient be held in the laboratory?

a. 12 hours.
b. 24 hours.
c. 36 hours.
d. 48 hours.

55. (237) If necessary, when ABO group and Rh-type specific cannot be given due to insufficient time, what blood may be released from the blood bank?

a. Group O Rh-negative blood with at least 50 percent of the plasma removed or free of hemolytic anti-A or anti-B.
b. Group O Rh-positive blood with at least 50 percent of the plasma removed or free of hemolytic anti-A or anti-B.
c. Group O Rh-negative blood with at least 70 percent of the plasma removed or free of hemolytic anti-A or anti-B.
d. Group O Rh-positive blood with at least 70 percent of the plasma removed or free of hemolytic anti-A or anti-B.

56. (238) In the preliminary investigation of an incomplete crossmatch, which of the following is not rechecked?

a. ABO group and Rh type of incompatible donor.
b. ABO group and Rh type of incompatible recipient.
c. Prior sample numbers against donor units.
d. Donor record for correct results in antibody screening.

57. (238) Which of the following is not a technical cause of incompatible crossmatching?

a. False positive reactions due to dirty glassware.
b. False positive reactions due to bacterial contamination.
c. Error in identification of the recipient's specimen.
d. Error in reading false positive reactions due to fibrin clots.

58. (239) Which of the following reactions should be primarily considered when fever and chills complicate transfusion of platelets which have been stored at room temperature?

a. Anaphylactic and allergic.
b. Bacterial.
c. Nonimmune hemolysis.
d. Delayed hemolytic.

59. (239) Since no practical laboratory tests are presently available to exclude infective donors, a careful history must be relied upon to prevent the transmission of which of the following diseases?

a. Syphilis.
b. Cytomegalovirus (CMV).
c. Viral hepatitis.
d. Malaria.

60. (239) In which of the following diseases is the incidence of the carrier state higher in commercial donors that in voluntary blood donors?

a. Syphilis.
b. Viral hepatitis.
c. Malaria.
d. Cytomegalovirus (CMV).

61. (240) Hemolysis may occur when blood is subjected to temperatures greater than

a. 25° C.
b. 30° C.
c. 35° C.
d. 40° C.

62. (241) What should be your first step in the investigation of a transfusion reaction?

a. Recheck pre-transfusion blood of recipient.
b. Recheck post-transfusion blood of recipient.
c. Recheck for clerical errors.
d. Recheck pilot samples of donor blood.

63. (239-242) Which of the following statements concerning hepatitis B associated antigen (HB_sAg) is not correct?

a. It is a type B virus.
b. It causes infectious hepatitis.
c. It is serum hepatitis.
d. It is designated MS-2.

64. (243) Red blood cells are coated with HB_sAb and in the presence of HB_sAg, the cells will be agglutinated in which of the following procedures?

a. Latex agglutination (LA).
b. Counterelectrophoresis (CEF).
c. Reverse passive hemagglutination (RPHA).
d. Reverse passive latex agglutination (RPLA).

65. (243) The RIA and RPHA method are classified as which of the following types of hepatitis tests?

a. First generation.
b. Second generation.
c. Third generation.
d. Fourth generation.

66. (244) In maintaining quality assurance in the blood bank it is possible to locally apply strict controls in all of the following areas except

a. human factors or variables.
b. physical equipment.
c. reagents and solutions.
d. manufacturing techniques.

67. (245) Packing in a centrifuge is a factor of

a. time and speed.
b. time and viscosity.
c. viscosity and volume.
d. volume and speed.

68. (245) How much longer does it take to bring suspensions of red cells and serum to 37°C. in a dry bath than in a water bath?

a. 1 minute.
b. 5 minutes.
c. 10 minutes.
d. 15 minutes.

69. (246) The speed and intensity with which an antigen and antibody react may best be explained by their

a. specificity.
b. reactivity.
c. avidity.
d. selectivity.

70. (246) The minimum federal requirements for anti-human serum is the ability to react with which of the following weak antigens?

a. Rh_o(D).
b. rh'(C).
c. hr'(c).
d. hr"(e).

71. (247) Accidental injury to a donor having a low hemoglobin usually can be prevented if which of the following actions is taken?

a. The blood donor record card is checked.
b. The blood is collected more slowly.
c. Less than a full unit is collected.
d. A good venipuncture is made.

72. (248) What is the minimum weight at which a donor may give 450 ±45 ml of blood in addition to pilot tubes not exceeding 30 ml?

a. 70 lbs (31.8 kg).
b. 90 lbs (40.9 kg).
c. 110 lbs (50 kg).
d. 130 lbs (59.1 kg).

73. (248) A female donor should have a hemoglobin level of at least

a. 11.5 gms.
b. 12.0 gms.
c. 12.5 gms.
d. 13.5 gms.

74. (249) Healthy civilian donors are acceptable if they are within the age bracket of

a. 17-40.
b. 18-56.
c. 18-66.
d. 17-66.

75. (249) Immunization for rabies will disqualify a donor for

a. six months.
b. two weeks.
c. one week.
d. one year.

76. (249) Oral contraceptives, mild analgesics, minor tranquilizers, vitamins, or weight reduction pills are normally

a. causes for both deferment and later rejection.
b. not causes for deferment or rejection.
c. causes for rejection.
d. causes for deferment.

77. (250) All of the following donors are permanently excluded except

a. donors with a history of viral hepatitis at any time.
b. donors who are or have been a drug addict.
c. donors who have ever had a confirmed positive test for HB_sAg.
d. inmates of penal or mental institutions.

78. (250) All of the following donors should be deferred for at least six months except

a. donors having recently received skin allographs, tattoos, or ear piercing.
b. recipients of blood components such as fibrinogen, AHG concentrates or immune vaccines.
c. donors who have had close contact with a patient with viral hepatitis.
d. donors whose unit of blood was administered to a recipient who developed post-transfusion hepatitis within six months.

79. (251) An advantage of plastic bags over glass containers as blood collecting containers is that they are less damaging to

a. plasma.
b. thrombocytes.
c. erythrocytes.
d. leukocytes.

80. (252) The phelobotomist who repeatedly asks such questions as, "Are you sure you feel all right?" or "Let me know when you feel sick," is

a. really concerned about his donor.
b. psychologically encouraging the donor to forget what's going on.
c. psychologically encouraging the donor to react.
d. able to keep the donor's mind at ease.

81. (253) Recommended materials, in proper order, for cleaning a blood donor's arm are surgical soap,

a. 10 percent acetone in 70 percent alcohol, and tincture of iodine.
b. tincture of iodine, and 10 percent alcohol in 70 percent acetone.
c. 10 percent alcohol in 70 percent acetone, and tincture of iodine.
d. tincture of iodine, and 10 percent acetone in 70 percent alcohol.

82. (253) If the donor becomes violently ill while you are taking his blood, which of the following actions should you take first?

a. Cover his forehead with a wet towel.
b. Use an ammonia inhalant.
c. Elevate his feet.
d. Withdraw the needle.

83. (254) What must be done first when starting the phlebotomy?

a. Have the donor open and close the hand.
b. Inspect the bag for any defects.
c. Uncover the sterile needle and make a visual check.
d. Inspect the site of the intended venipuncture for any reactions.

84. (254) Immediately after blood begins entering the collection bag, the technician should

a. mix the blood and anticoagulant slowly.
b. check the tube and bag numbers.
c. agitate the bag rapidly.
d. check the donor card.

85. (255) When the pH of the blood in storage falls, what essential process of red cell metabolism is affected?

a. Sodium and potassium ions approach an equilibrium.
b. There is a decreased affinity of the red blood hemoglobin for oxygen.
c. Enzymes necessary for the phosphorylation of glucose are inhibited.
d. Enzymes necessary for the phosphorylation of glucose are enormously increased.

86. (255) Blood collected in heparin as an anticoagulant must be used within what allowable time?

a. 24 hours.
b. 48 hours.
c. 7 days.
d. 21 days.

87. (256) Blood should be stored at a temperature of 1° C. to 1° C. and this temperature should vary no more than

a. 1° C.
b. 2° C.
c. 5° C.
d. 6° C.

88. (256) How often must the temperature charts from the mechanical recording device on the blood bank refrigerator be changed?

a. Daily.
b. Weekly.
c. Monthly.
d. Bimonthly.

89. (257) What two basic advantages does frozen deglycerolyzed blood have over liquid banked blood for dialysis patients?

a. Reduction of hepatitis infectivity and HL-A antigenicity.
b. Elimination of plasma antibodies and microemboli in donor blood.
c. Elimination of plasma antibodies and platelets.
d. Reduction of hyperkalemia and anticoagulant toxicity.

90. (258) In the high-glycerol, slow-freezing method for the cryopreservation of red cells, a positive anti-globulin test due to the low ionic strength of the medium is prevented by what constituent in the medium?

a. Glycerol.
b. CPD.
c. EDTA.
d. Glucose.

91. (258) In the high-glycerol slow-freezing method for the cryopreservation of red cells, before cells to be deglycerolyzed are washed, they are first diluted with which of the following solutions?

a. 10 percent glucose.
b. 5 percent fructose.
c. 50 percent glucose.
d. 10 percent fructose.

92. (258) What medium is used to lower the temperature of blood very quickly when using the rapid-freeze method?

a. Dry ice.
b. Helium.
c. Liquid oxygen.
d. Liquid nitrogen.

93. (259) When blood is shipped within the United States, the receiving unit should be notified of the time of arrival by

a. registered mail.
b. messenger.
c. telephone.
d. airmail.

94. (259) Blood should be shipped so that it will arrive at the receiving unit within

a. one day.
b. two days.
c. three days.
d. four days.

95. (260) Air Force medical facilities normally may obtain blood for transfusion from all of the following sources except

a. volunteer donors on military bases without payment.
b. the American Red Cross in the US (Puerto Rico, Alaska, and Hawaii).
c. civilian blood banks licensed by the FDA.
d. paid professional donors.

96. (260) The responsibility to provide blood donors at the frequency and in the quantity needed, and to meet contingency requirements for blood by Air Force hospitals is primarily at which of the following levels?

a. Base.
b. Command.
c. Medical unit.
d. Medical laboratory.

97. (261) What office is responsible for the National Blood Program?

a. The Office of Emergency Planning.
b. The Military Blood Program office.
c. The Office of the Surgeon General, USAF.
d. The Office of the Assistant Secretary of Defense.

98. (262) After giving blood, the donor should remain in a supervised recovery area for at least

a. 10-15 minutes.
b. 20-30 minutes.
c. 30-45 minutes.
d. 1 to 2 hours.

99. (262) In the routine processing, if the blood is typed as $Rh_o(D)$ negative, what additional test should be done?

a. Test for D^u variants only
b. Test for D^u variants and C (rh').
c. Test for D^u variants and E (rh").
d. Test for D^u variants and c (hr').

100. (263) When notified by the Military Blood Program Office of the need to support a contingency or mobility operation, what agency is responsible for quality control, double checking the group and Rh, repacking, and shipment of blood to bases requesting blood?

a. Blood Donor Units, Wilford Hall Medical Center.
b. Armed Services Whole Blood Processing Laboratory.
c. The Office of Emergency Planning.
d. The American Red Cross.

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CDC 90413

MEDICAL LABORATORY TECHNICIAN—HEMATOLOGY, SEROLOGY, BLOOD BANKING AND IMMUNOHEMATOLOGY

(AFSC 90470)

Volume 3

Serology

Extension Course Institute

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MCMXLVII
UNITED STATES OF AMERICA

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EXTENSION COURSE INSTITUTE, GUNTER AIR FORCE STATION, ALABAMA

THIS PUBLICATION HAS BEEN REVIEWED AND APPROVED BY COMPETENT PERSONNEL OF THE PREPARING COMMAND IN ACCORDANCE WITH CURRENT DIRECTIVES ON DOCTRINE, POLICY, ESSENTIALITY, PROPRIETY, AND QUALITY.

Preface

THIS THIRD volume of CDC 90413 is concerned with clinical serology. Chapter 1 discusses concepts of the immune response and the interaction of antigen and antibody. The special reactions that produce agglutination as an end result are discussed in Chapter 2. Latex-fixation, precipitin, and anti-streptolysin tests are presented in Chapter 3. The precipitin reaction, with reference to immune diffusion techniques, is discussed in detail and a new test for streptococcal exoenzymes is introduced. A short historical review of syphilis and a discussion of various serological tests for syphilis are presented in Chapter 4.

A glossary of technical terms used in Volumes 1, 2, and 3 of this CDC is shown at the back of Volume 3.

Please note that in this volume we are using the singular pronoun *he, his,* or *him* in its generic sense, and not its masculine sense. The word to which it refers is person.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to School of Health Care Sciences/MSTW, Sheppard AFB TX 73611. NOTE: Do not use the suggestion program to submit corrections for typographical or other errors.

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This volume is valued at 30 hours (10 points).

Material in this volume is technically accurate, adequate, and current as of June 1977.

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NOTE: In this volume, the subject matter is developed by a series of Learning Objectives. Each of these carries a 3-digit number and is in boldface type. Each sets a learning goal for you. The text that follows the objective gives you the information you need to reach that goal. The exercises following the information give you a check on your achievement. When you complete them, see if your answers match those in the back of this volume. If your response to an exercise is incorrect, review the objective and its text.

Basic Principles of Immunology and Serology

THE EARLIEST recognition of the immune phenomenon was associated with smallpox. Thousands died as a result of this and other deadly diseases. Nevertheless, the fact that those persons who had recovered from an attack of smallpox did not become reinfected led orientals to practice self-inoculation with smallpox matter. As you may recall, it was Jenner who undertook a concise approach to vaccination by vaccinating individuals with cowpox.

Today we know that our bodies produce substances that protect us against reinfection. We also know that the agent causing the disease stimulates our body to produce these protective substances. In the laboratory it is not always possible to isolate and identify the infecting microorganism, but we can usually study the body's response to infection and thereby gain an indication of the nature of the causative agent.

In this chapter we shall concern ourselves with an infected person's defensive mechanism against an infectious agent. This requires an understanding of natural and acquired defenses and the laboratory tests used to monitor the patient's response. Your study of immunity is an important contribution which aids the physician in diagnosis and treatment of infectious diseases.

1-1. Mechanics of Immunity

When a person comes into contact with microbes which cause an infectious disease, his body responds in an attempt to counteract the effect of the disease-producing agents. If the response is such as to provide partial or complete protection, then that person is said to have a certain degree of immunity. Immunity, which may be either natural or acquired after birth, is the subject matter of the science of immunology.

400. Define immunology and cite the other given types of immune responses.

Immunological Concepts. Historically, immunology was defined as "that body of knowledge concerned with the biological, chemical, and physical factors that contribute to the body's resistance to immunogenic agents." Immunogenic agents are those bacteria, viruses, and other substances that are capable of causing the body to produce protective substances called antibodies. We generally refer to these immunogenic agents as antigens. Primarily, immunologists were concerned solely with the observation that certain individuals who recovered from a particular infectious disease would not again contract the same disease and that this same resistance to many diseases could be induced through vaccination. As knowledge advanced, it was discovered that the immune response must also include the state of hypersensitivity, the rejection of allografts (recipient receives tissue or organ graft from a genetically dissimilar donor of the same species), grafts versus host reactions, and other phenomena related to our intolerance of many things foreign when they occur within our bodies. Thus, the immune response must not just be limited to include infectious organisms or their products.

You are probably familiar with various expressions of immunity. It has almost become a habit for us to associate immunity with infectious processes caused by bacteria, viruses, or rickettsial organisms. However, you should not lose sight of the fact that immunological responses are very often caused by agents other than living microorganisms. Many substances foreign to the body are capable of being antigenic. In fact, microbial substances make up only a small percentage of immunogenic agents. Specific antibodies or hypersensitivity may be elicited by an almost limitless number of other substances such as pollens, foreign serum proteins, venoms, and others. Usually these antigens are protein in composition, but polysaccharides, lipids, and other chemical entities may also stimulate an immune response. You should now be aware of the fact that immunology now encompasses many aspects which are quite different from the original concept of resistance to infectious disease.

Exercises (400):

1. Define the original concept of immunology.

2. Are all bacteria and viruses immunogenic agents?

3. In immunology, what other types of reactions must also be included under the immune responses?

4. In addition to microbial organisms such as bacteria, viruses, and rickettsia, what other immunogenic agents may elicit antibodies in hypersensitivity?

5. What other substances besides proteins may stimulate an immune response?

401. Give the meaning of serology and list some of the other diagnostic uses of serology.

Serology. Serology is literally the study of serum. More specifically, serology is diagnosis through the use of detection of serum globulins known as antibodies. These antibodies may even have been produced in another animal and then used to diagnose disease in man. In effect, any in vitro diagnostic tests using antibodies can be called a serological test or serotest. However, antibodies are only half of the reactants in serotests. Antibodies must have something to react with, namely, antigens.

Today, diagnostic uses of serology extend beyond the identification of microorganisms or the measurement of antibodies in infectious disease. It is now possible to detect and measure antibodies in autoimmune disease, to perform immunoassay of hormones and immunoglobulins, to improve prognosis in organ or tissue transplants by determining the compatibility level between the recipient and donor, to detect certain tumor antigens, and to aid in many other diagnoses of both infectious and noninfectious diseases.

Exercises (401):

1. Define serology.

2. List some of the other diagnostic uses of serology.

402. Define natural resistance to disease and cite the manner in which the resistance may vary in different animal species.

Natural Resistance. When we speak of natural resistance to disease we are referring to immunity dependent upon some special property of a particular animal species rather than to a specific antibody. For instance, the fact that we are human gives us a certain nonsusceptibility that is different from that of the lower animals. Conversely, many animals have a natural resistance quite different from that of human beings. We know, for example, that foot-and-mouth disease rarely affects man, whereas it produces a fatal infection in cloven-hoofed animals. Tuberculosis is common in humans, cattle, pigs, and fowl, but it is uncommon in sheep, cats, dogs, and horses. Coldblooded animals such as snakes and turtles are not susceptible to gonorrhea, mumps, typhoid fever, measles, and many other important human diseases.

Exercises (402):

1. What is natural resistance?

2. Why don't humans suffer from foot-and-mouth disease in the same manner as cows?

403. Indicate whether the given statements correctly reflect the concept of innate immunity.

Innate Immunity. Innate immunity may be described as the capacity a normal organism possesses in order to remain relatively unharmed by agents which are harmful and are present in its environment. It is brought about by physiologic and anatomic features associated with the species and does not depend upon antibodies which can be detected by ordinary methods. The concept of nonsusceptibility is borne out, however, by the occurrence of infections in some species but not in others. For example, man will not be infected by a large number of animal pathogens such as distemper, hog cholera, or cattle plague. On the other hand, many human diseases such as cholera, dysentery, measles, syphilis, or mumps do not affect lower animals. Coldblooded animals are nonsusceptible to tetanus toxins, perhaps because some physiologic factors such as body temperature and diet contribute to nonsusceptibility. Each species has innate immunity to that particular disease. There are many degrees of innate immunity displayed. All species do not possess the absolute immunity described in the example. Even members of the same species vary in their ability to resist a given disease.

Exercises (403):

Indicate whether each of the following statements is true (T) or false (F). If you indicate false, explain your answer.

T F 1. In innate immunity, a normal organism possesses certain antibodies enabling it to remain relatively unharmed by harmful agents in the environment.

T F 2. The concept of nonsusceptibility is borne out by the occurrence of infections in some species but not in others.

T F 3. In innate immunity, man will be infected by a large number of animal pathogens such as distemper, hog cholera, or cattle plague.

T F 4. Human diseases such as cholera, dysentery, measles, syphilis, or mumps do not affect lower animals.

404. Identify the anatomical and chemical defense mechanisms, excluding antibodies, in terms of the given characteristics and features.

Defense Mechanisms of Innate Immunity. Many defense mechanisms are active in innate immunity. Although there is much overlapping, these defense mechanisms can be divided into two categories: anatomical and chemical, excluding antibodies.

Anatomical defense mechanisms. Anatomical defense mechanisms include the following:

a. Skin. The intact skin is an effective physical barrier to most disease-causing organisms. In addition, chemical mechanisms also operate here because the acid pH of the surface of the skin inhibits many microbes.

b. Phagocytes. Throughout the body there is found a wide variety of cells which actively engulf (phagocytize) foreign particles, including microbes. White blood cells are well-known as phagocytes. Kupfer cells of the liver and microglial cells of the brain are examples of phagocytes which are localized in specific organs. As usual, chemical mechanisms cannot be excluded, for even the actual attraction of the phagocyte to the foreign particle is chemically or ionically mediated. Also, chemical degradation of the microbe takes place in the phagocyte if the phagocyte has the appropriate enzyme systems to destroy that particular microbe.

c. Mucus. Mucus may act as a physical "trap" for disease agents. In combination with other mechanisms such as the cough reflex and cilia of the respiratory tract, mucus becomes an effective factor in preventing disease. Mucus can also contain phagocytes, enzymes, and antibodies which add effectively to disease resistance.

d. Mouth. The simple act of swallowing moves potential pathogens to the stomach where few can survive. Coughing followed by swallowing aids in the removal and destruction of respiratory pathogens. Many enzymes and other microbial inhibitors are active in the salivary juices of the mouth too.

e. Urinary tract. The flushing effect of the irinary tract helps to physically remove potential pathogens.

f. Eyes. The flushing action of tears, along with the presence of enzymes in the tears, provides one of the best examples of innate immunity. Acquired immunity is of minimal importance here. Even so, the eyes are perhaps the most healthy organs of the body when one considers infectious disease.

g. Lymphatic system. This system of ducts running throughout the body has nodes at intervals. These nodes can physically filter microbes from lymphatic fluid. Also, the nodes are the sites of accumulation of white blood cells, including fixed tissue macrophages. These cells are then in an ideal position to phagocytize the offending microorganisms.

Chemical defense mechanism, excluding antibodies. The degree of immunity is also influenced by the following chemical defense mechanisms, excluding antibodies:

a. Lysozyme. This enzyme is present in many body fluids, such as mucus, tears, and saliva. Primarily, it attacks the cell walls of gram-positive bacteria.

b. Interferon. This protein is produced by the body in response to a viral infection or after the injection of dead viruses. It differs from antibodies in that interferon may provide protection against challenge by another completely unrelated virus while antibodies normally act only against the specific injected virus.

c. Properdin. This high-molecular-weight protein is effective against certain viruses and also against some gram-positive bacteria and even a few gram-negative ones. Properdin might be considered to be an antibody-like substance, since its action requires the presence of complement and magnesium ions, both of which are active in certain antibody reactions. In fact, properdin has been called a "natural antibody," but it lacks the specificity of an antibody and attacks many different organisms.

d. Inflammation. Invasion by certain microbes causes inflammation which brings several defense mechanisms into action. For one, the temperature at the site of the inflammation or even throughout the body may rise, thus becoming a less favorable environment for many pathogens. The increased permeability of blood vessels at the site allows antimicrobial factors and white blood cells to readily contact the infectious agent. Alteration of the chemical composition at the site also acts as a defense mechanism. For example, the pH goes down as lactic acid and carbon dioxide accumulate. All of these changes during inflammation combine to resist further reproduction and dissemination of the pathogen.

Exercises (404):

Match each defense mechanism in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. Chemical mechanisms also operate in this area because of its acid pH which inhibits many microbes.
___ 2. Kupfer cells of the liver and microglial cells of the brain are examples of these types which are localized in specific organs.
___ 3. The attraction of these factors to the foreign particles is chemically and ionically mediated.
___ 4. With the appropriate enzyme system present, chemical degradation of the microbe takes place in these structures.
___ 5. May act as a physical "trap" for disease agents.
___ 6. Can also contain phagocytes, enzymes, and antibodies which add effectively to disease resistance.
___ 7. Many enzymes and other microbial inhibitors are active in the digestive juices of this factor.
___ 8. The flushing effect of this mechanism helps to physically remove potential pathogens.
___ 9. Are perhaps the most healthy organs of the body when one considers infectious disease.
___ 10. This factor contains nodes that physically filter microbes from the fluid it contains.
___ 11. This substance is present in many body fluids such as mucus, tears, and saliva.
___ 12. This substance is produced by the body in response to a viral infection or after the injection of dead viruses.
___ 13. A high-molecular weight protein that is effective against certain viruses and also some gram-positive and a few gram-negative bacteria.
___ 14. Has been called a "natural antibody," but it lacks the specificity of an antibody and attacks many different organisms.

Column B

a. Lysozyme.
b. Eyes.
c. Skin.
d. Mucus.
e. Lymphatic system.
f. Mouth.
g. Interferon.
h. Urinary tract.
i. Properdin.
j. Phagocytes.

405. Define active immunity in terms of its function and characteristics and cite examples of both naturally-acquired active and artificially-acquired active immunity.

Acquired Immunity. This type of immunity is obtained or develops after birth. A baby derives immune substances to protect it in breast milk from its mother. Typhoid fever infection usually confers resistance to reinfection if the patient lives. In both these instances immunity is gained in the form of antibodies. In the first instance they were produced by the mother and incidentally or passively given to the baby. In the second instance the typhoid infection causes the patient's body to actively respond and produce antibodies. There are important differences between these two types of acquired immunity.

Active Immunity. Active immunity is an acquired immunity because it involves specific antibodies against a microorganism or foreign substance. It is called active immunity because the individual actively produced these antibodies himself at some stage in his life. The antibodies may arise as a result of a clinical or subclinical infection by the given microbe. They may also be produced in response to vaccinations with killed or attenuated organisms. In either case, the individual produces the antibodies. Antibodies produced following actual infection are said to confer naturally-acquired active immunity. Vaccination produces artifically-acquired active immunity. The resulting antibodies may contribute to immunity in several ways or not at all. Some may cause a microbe to be more readily phagocytized, destroy it directly, neutralize some toxin produced by the organism, or combine these actions. Man many also produce antibodies that have no apparent protective value at all but their detection may be useful diagnostically.

Active immunity is not always perfect and may be graded into a series of levels that extend from complete immunity to a state approaching complete susceptibility. Since the body's defenses can often be overcome if the challenging dose of microbes is large enough, several grades of illness may occur between the two extremes. Figure 1-1 illustrates the levels of immunity. In this illustration resistance becomes progressively lower as you move from a high level of immunity (heavy stippling) to an absence of immunity (no stippling).

Naturally acquired active immunity. The fact that a person has recovered from an infectious disease does not guarantee resistance to another attack by the same microbe. For example, influenza and gonorrhea result in very short-lived immunity and repeated attacks are common. On the other hand, a single infection of measles or chicken pox usually confers life-long immunity to reinfection. There are other examples of these extremes of immunity and also many that fall between the extremes.

Especially significant to the serologist is the rise in antibody titer during the course of a disease. In many cases, serological tests are the only practical diagnostic tools available. Perhaps isolation of the causative organism would be impossible, too expensive, or take too long to benefit the patient.

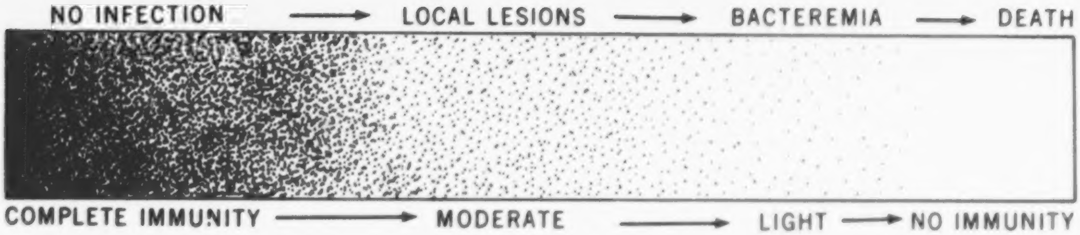

Figure 1-1. Levels of immunity.

Another important aspect of serological testing is the determination of antibody levels at a given time. A good example of this is the testing of serum from pregnant females for antibody titers against rubella (German measles). In this case, a certain woman is immune to the rubella virus. Consequently, the safety of the developing fetus will most likely be assured in the event the immune woman should become exposed to rubella.

Artificially acquired active immunity. For many diseases, vaccination has proven to be an effective method of providing immunity. This immunity is also not absolute and the relative efficiency of each vaccine can be guessed by the frequency that reimmunization is required. In endemic areas, cholera and plague shots are given every 6 months; yellow fever shots, every 10 years. Obviously, the yellow fever vaccine is much more effective than cholera and plague vaccines. Although antibodies produced as a result of vaccination may benefit the patient, these same antibodies usually confuse the seriologist. His tests usually will not differentiate between artificially acquired antibodies and those produced in the presence of active disease. Consequently, a negative history with a rising antibody titer becomes very important in serodiagnosis. Of course, the problem is minimized in diseases where vaccination is not practiced.

Exercises (405):

1. Define acquired immunity.

2. Which type of acquired immunity (passive or active) is accomplished after a typhoid infection has conferred resistance to reinfection of the disease if the person lives?

3. Why is active immunity considered as such?

4. When antibodies are produced following actual infection, they are said to confer what type of immunity?

5. What type of immunity is produced by vaccination?

6. Give two examples of diseases that produce a short-lived immunity and two examples of those producing life-long immunity.

7. What condition is especially significant to the serologist during the course of the disease?

8. In testing for cholera or plague antibodies, how does the serologist differentiate between artifically acquired antibodies and those produced in the presence of active disease?

406. Cite the difference between active acquired and passive acquired immunity and show examples reflecting the difference between naturally acquired passive immunity and artificially acquired passive immunity.

Passive Immunity. Passive immunity is another type of acquired immunity because antibodies are involved. It differs from active immunity by the fact that the antibodies are produced in another individual or animal and then injected into the recipient. The recipient passively receives the immunity conferred by these antibodies. Although passively-transferred, antibodies are important to the recipient; they are short-lived and rarely assayed by the clinical serologist. As with active immunity, passive immunity may be acquired both naturally and artificially.

Naturally acquired passive immunity. This type of immunity is significant mainly in the survival of the newborn infant. The infant passively acquires antibodies from its mother. The antibodies may pass from the immune mother to the fetus across the placental barrier. In addition, the infant may acquire these antibodies from its mother's milk which is rich in antibodies for a short time after birth. Of course, immunity is transferred only if the mother is immune to a given disease. Passive immunity is especially important to the newborn because they are incapable of producing antibodies of their own for a few months after birth. The antibodies received via natural transfer from the mother are relatively short-lived with protection seldom exceeding about 6 months. Fortunately, by this time the infant's immunological system is fully functional.

Artifically acquired passive immunity. Antibodies that have been produced in another individual or animal and then administered by injection to the recipient provide this type of immunity. This method has been used extensively in the past in the treatment of diphtheria and tetanus through the injection of antibodies produced in horses. Before the advent of antibiotics, passively-administered antibodies were used as the treatment for pneumococcal pneumonia. Currently, passive immunization is mainly used for prophylaxis following exposure to such diseases as rubella and infectious hepatitis. This is usually accomplished by injecting the recipient with antibodies (gamma globulin) which have been extracted from the blood of immune persons. These antibodies provide protection for a relatively short time and then are removed from the system.

The immunity gained by passive immunization is usually temporary. It protects immediately after injection and for a short time thereafter. The procedure is particularly useful in providing protection in the critical periods of infancy and early childhood. Passive immunizing agents are not usually thought of as being themselves immunogenic. That is, they do not elicit antibody response against the infectious agent they are given to protect against. However, since they are produced in other animals, injections of serums from these animals can result in sensitization. For example, frequent injections of horse serum will cause sensitization and result in serum sickness.

Exercises (406):

1. How does active immunity differ from passive immunity?

2. In naturally acquired passive immunity, how does the fetus acquire antibodies from its mother?

3. Why is passive immunity especially important to the newborn?

4. Currently, for what purpose is passive immunization mainly used?

5. How is immunization accomplished in methods of passive immunization?

6. Why is passive immunity of short duration?

1-2. Antigen-Antibody Interaction

Much is known today about antigens, antibodies, and their reactions. Scientific study of these entities began in the latter part of the 19th century. While most of the early work concerned bacteria and their antigens, later research has taken great strides forward in blood transfusion technology. For the most part, these fields of study were separate endeavors for many years. Consequently, the immunohematologist developed definitions quite different from those used by the bacteriologist. Only recently have we begun to understand that serological reactions encountered in the two fields are expressions of a fundamental occurrence—the antigen-antibody reaction.

407. Define an antigen and identify its properties and characteristics in terms of their purposes and functions.

Antigens. To understand why antigens react as they do, we must first define what they are and then discuss their unique properties. The word antigen is derived from the Greek words *anti* (against) and *gennen* (to produce). Any foreign substance that stimulates the body (or any animal's body) to produce antibodies is called an antigen. To qualify as an antigen, these foreign substances must also react in some specific way with the antibodies that have been produced. An antigen might be a microbe, part of a microbe, some microbial toxin, or even some foreign product completely unrelated to microorganisms and the disease they cause. Antigens stimulate the formation of specific antibodies. These antibodies must react with the antigen in some observable way.

Characteristics of Antigens. Although antigens comprise a wide variety of substances, they have several common charateristics. These characteristics and unique properties all function together to qualify a substance as an antigen. Let's review the following characteristics.

Foreign to the body. Normally, the body does not respond by producing antibodies against itself. A notable exception to this is the production of antibodies against the lens of the eye. This may be explained by the fact that the lens is not in intimate contact with the reticuloendothelial system which produces the antibodies, so the lens is, therefore, recognized as foreign. In addition, there are other examples of so-called autoimmune diseases in which the body produces antibodies against itself, but these are usually associated with some malfunction of antibody production or antigen recognition. In general, however, the reticuloendothelial system responds only to antigens that are foreign, but even members of the same species have many antigens that are foreign to other members of the species. In man, the red blood cell antigens with their significance in blood transfusions and erythroblastosis fetalis provide good examples of foreignness within a species. Another example is seen in kidney transplantations, where usually only kidneys from very close relatives are accepted by the recipient. In time, kidneys from unrelated donors are usually rejected due to reaction with antibodies produced against kidney antigens. However, the mere fact that a substance is foreign to an individual does not confer antigenicity. Other characteristics must be present also.

Molecular weight. Another characteristic of antigens is that they are generally complex chemicals of very high molecular weight. A molecular weight of 10,000 is considered to be the minimum, but antigens with a molecular weight down to about 3,000 have been known. Some antigens have molecular weights in the millions. In general, the strongly antigenic substances have higher molecular weights than weakly antigenic substances.

Chemical nature. Most antigens are proteins or proteins complexed with carbohydrates or lipids and, as such, are derived from living organisms. As a general rule, carbohydrates and lipids are not antigenic unless in a protein complex. Only humans and mice appear to be able to produce antibodies against pure carbohydrates, such as certain polysaccharides, but this is a rare occurrence in nature.

Each antigen has two parts—a carrier and a determinant. The carrier is usually a protein. Aside from contributing most of the molecular weight and increasing the rigidity of the antigen, the carrier functions mainly as its name implies—a carrier. The determinant is the important part of an antigen. It makes an antigen a specific, foreign substance. Although the determinant cannot stimulate antibody production by itself, it can react with the antibodies once they are produced. A determinant functioning in this fashion without its normal carrier is called a hapten. Many consider determinants and haptens as synonyms.

Determinants (or haptens) comprise only a small portion of the total antigen molecule. For example, even the stereoisomers, D-tartaric acid and L-tartaric acid (M.W. 150), when conjugated with a carrier protein, form specific antigens. Each stimulates the production of antibodies which react only with the given isomer. In addition to lending specificity to the antigen and stimulating the body to produce specific antibodies against them, determinants are the reaction sites for the antibodies in antigen-antibody reactions. Each antigen molecule usually has about a dozen of these reaction sites (determinants), but some molecules may have a hundred or more. By contrast, the most common type of antibody molecule has only two reaction or combining sites while other types may have up to ten sites. Therefore, most antibodies are said to be bivalent, while antigens are multivalent. The valence of antigens and antibodies is especially important to the serologist in mixing the proper proportions of each in serologic tests.

Reactivity with antibodies. The last characteristic that an antigen must possess is that it must be able to react in some demonstrable way with the antibodies which have been produced in response to that antigen. The utilization and observation of these antigen-antibody reactions provide the basis for serological diagnosis and are covered later in this chapter and throughout this volume.

Fate of Antigens. Antigens may enter the body in a variety of ways. They may be acquired during the normal course of living, during disease, or following vaccinations. They may also be injected accidentally such as during a blood transfusion. Once they enter the body, they are very rapidly localized in the fixed macrophages of the liver, spleen, and bone marrow. The macrophages apparently process the antigen in some way, but the lymphocytes actually produce the antibodies. Some antigens are physically present in the macrophages during the time that the lymphocytes are actively producing antibodies. Whether this is necessary for continued antibody production is under investigation. Many antigens appear to be present for several months after injection; so retention may be essential for antibody production to occur.

Exercises (407):

1. Give a simple definition for antigen that will cover any kind of substance.

2. How can you explain antibody production against the lens of the eye?

3. In autoimmune diseases when the body produces antibodies against itself, what is usually the underlying malfunction?

4. What is the usual minimum molecular weight of an antigen?

5. What is the chemical composition of most antigens?

6. What are the two parts of an antigen?

7. What purposes do the determinants serve?

8. What characteristic of the antigen provides the basis for serological diagnosis?

9. What happens when the antigens enter the body?

10. Some ________ are physically present in the macrophages during the time that the ____________ are actively producing antibodies.

408. Indicate whether the given statements correctly reflect some properties and characteristics of antibodies.

Antibodies. Antibodies are also complex proteins. This fact is stressed here because it is so often emphasized that antigens are protein in nature, and we tend to dismiss the fact that antibodies are proteins too.

Just as antigens are defined in terms of their reactivity with antibodies, all antibodies are intimately associated with their antigens. These antibodies must be able to react in some demonstrable way with the antigen which stimulated their production. Antibodies of significance to the serologist are found in the serum (or plasma) fraction of blood, but there are some antibodies that remain fixed to certain tissue cells. These fixed antibodies are of minimal significance to the serologist although their importance to the health of the individual should not be discounted. Whether fixed to cells or free in the serum, antibodies have several characteristics in common.

Specificity. In general, each antibody will react only with the antigen that stimulated the body to produce that antibody. In other words, each antibody possesses a high degree of specificity. As with any biological system, there are exceptions to the rule. These exceptions will be discussed later under antigenic variation and as they apply to each test procedure.

Chemical nature. Antibodies are found in the globulin fraction of serum protein. More specifically, most antibody activity is in the gamma globulin fraction of serum globulin. Because these globulins are active in immunity, they are frequently called immunoglobulins as a synonym for antibodies.

Exercises (408):

Indicate whether each of the following statements is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. Antibodies, in contrast to antigens, are complex polysaccharides.

T F 2. Antibodies of significance to the serologist are found in the serum fraction of the blood.

T F 3. Each antibody possesses a low degree of specificity.

T F 4. Antibodies are usually found in the albumin fraction of serum.

T F 5. Immunoglobulins is a synonym for antibodies.

409. Point out the five classes of immunoglobulins in terms of the given functions and characteristics and state the methods used for analysis and classification.

Immunoglobulins. The immunoglobulins (antibodies) of man can be further classified based on the fact that they are antigenic themselves when injected into certain animals. The animals produce antibodies which precipitate the human immunoglobulins. Five main classes of immunoglobulins have been identified in man by this method. They are IgG, IgM, IgD, IgA, and IgE. Most antibody activity in human serum is due to IgG, IgM, or IgA with certain diseases causing the production of more of one class than another. For example, antibodies against bacterial endotoxins are primarily IgM, while those against the mumps virus are IgG. In addition, the class that is produced in greatest quantity varies with the number of times the individual has had contact with a particular antigen. Usually on the first contact, such as a vaccination, IgM is the main antibody produced; but on a second or subsequent contact, IgG usually predominates.

The five classes of immunoglobulins also vary somewhat in molecular weight and structure. All are very large molecules with most classes having a molecular weight of 150,000 to 200,000; however, the molecular weight of IgM is about 900,000. Each immunoglobulin molecule consists of a combination of two types of chains of amino acids. These chains have been designated as light chains and heavy chains. The most abundant immunoglobulin molecule, IgG, is made up of two light and two heavy chains. An IgM molecule has ten light and ten heavy chains. Each chain of the molecule has a region with a constant amino acid sequence and a variable region. The variable region is also the binding site in antigen-antibody reactions. Figure 1-2 shows a schematic structure of an antibody (IgG) molecule.

Analysis of Immunoglobulins. As laboratory procedures for studying proteins have advanced, other systems of classification have come into use. These systems are related to the physical characteristics of immunoglobulins—namely, their sedimentation and electrophoretic properties. We can separate serum protein into its components using the electrophoretic mobility of these components. Figure 1-3 shows a typical electrophoretic pattern of human serum. The two major components on this pattern are albumin and globulin. The globulins, which are of interest to us because of their immunological properties, can be further divided into three groups. These are alpha (α), beta (β), and gamma (γ) globulins. If the albumin and globulins are separated immunoelectrophoretically, fractions will separate in the order shown in table 1-1. These fractions can be eluted from agar gels or other media and tested for antibody activity.

Many methods for quantitative assessment of the immunoglobulins have been described. Three of these are currently of greatest value:

(1) Radial immunodiffusion (RID) with limited or with timed diffusion;

(2) Automated immune precipitation (AIP); and

(3) Electroimmunoassay (EIA), also called rocket technique.

The singly most powerful analytic procedure has been immunoelectrophoresis. Combining the techniques of electrophoresis and immunodiffusion, this method has served as a revolutionary stimulus to an increased understanding of the production of serum proteins. Not only does this technique provide a semiquantitative tool for the evaluation of serum proteins but also it allows for an assessment of the clonality of cells which produce a particular immunoglobulin. This means that varied dyscrasias in immunoglobulin synthesis may be distinguished and the appropriate therapeutic measures, in case of disease, may be instituted.

Immunoglobulins can also be separated in a procedure that takes advantage of the fact that different classes of antibodies have different molecular weights. In general, immunoglobulins have very high molecular weights (125,000 to 900,000); however, if macroglobulins are included, the figure is close to 1,000,000. Separation is accomplished in an ultracentrifuge at speeds of up to 70,000 r.p.m.

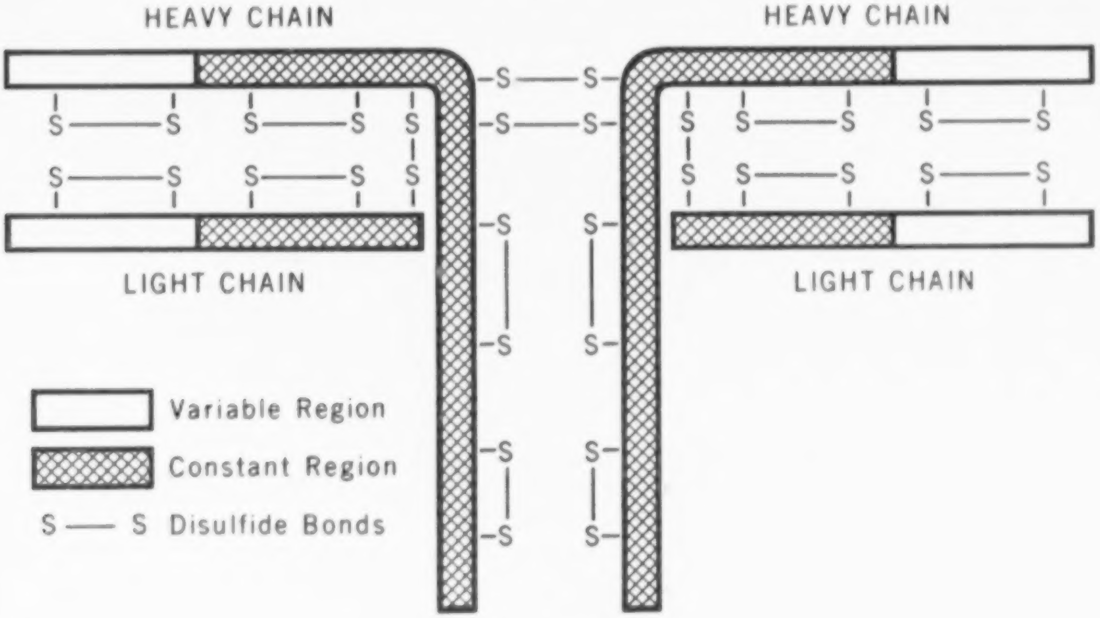

Figure 1-2. Schematic structure of an IgG antibody.

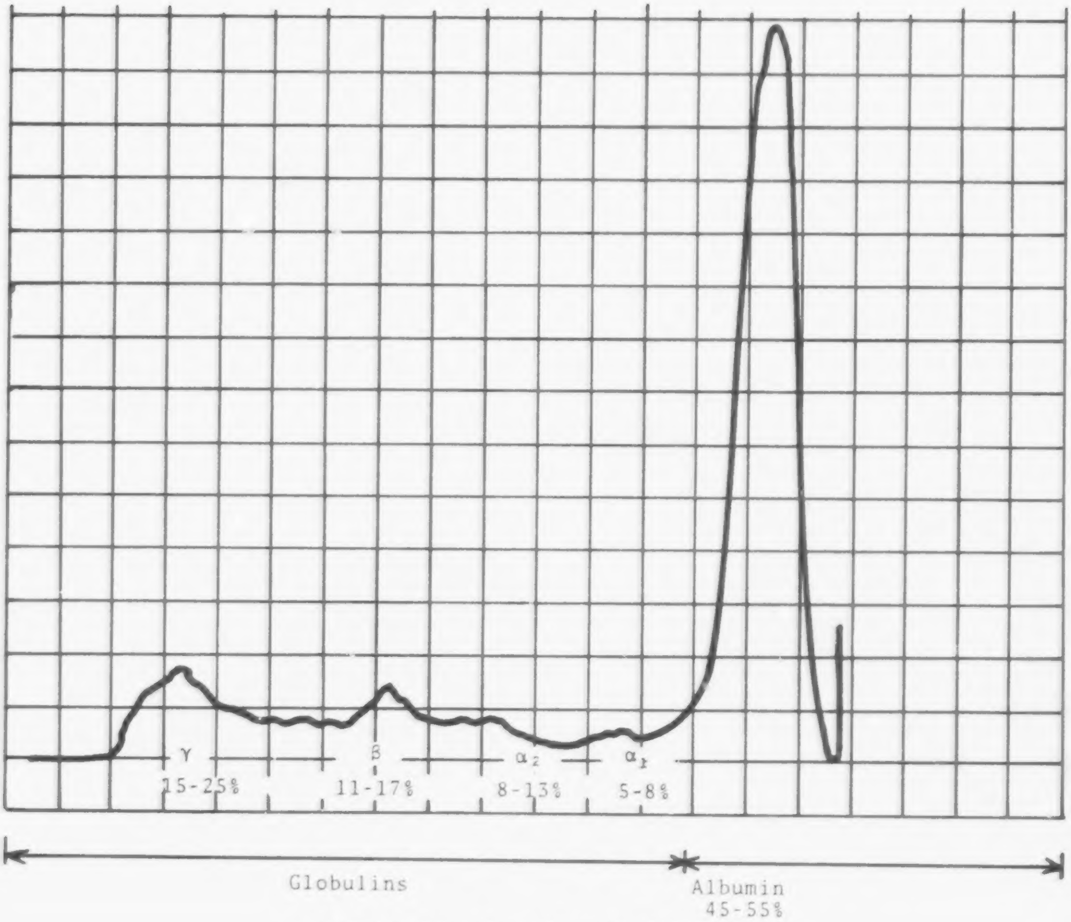

Figure 1-3. Human serum electrophoresis pattern.

TABLE 1-1
HUMAN SERUM COMPONENTS

Globulins

Albumin: $\overset{0}{\alpha_1}$ $\overset{1}{\alpha_1}$ $\overset{2}{\alpha_1}$ $\overset{3}{\alpha_1}$: $\overset{1}{\alpha_2}$ $\overset{2}{\alpha_2}$: $\overset{1}{\beta}$ $\overset{2}{\beta}$ $\overset{3}{\beta}$ $\overset{4}{\beta}$: γ γ_1 γ_2 γ_3 γ_4 γ_5: Specimen Applied Here

← Direction of Migration ←

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Solutions are placed in a quartz cell and are centrifuged. While the machine revolves, a photoelectric cell measures the density of the solution. The heaviest molecules, of course, sediment first, followed in order by increasingly lighter molecules. Thus the globulin solution is separated into bands containing molecules of varying densities. These bands are identified by a numeral followed by the letter S (2S, 7S, 19S, 20S, etc.). The S refers to the *sedimentation constant* or Svedberg (S) units. Analyses of globulins have shown that 7S fractions have a molecular weight of about 160,000 while 19S fractions weigh in at 900,000.

Both the electrophoretic and sedimentation constant methods of classifying immunoglobulins have been widely used.

Exercises (409):

1. What are the five identified classes of immunoglobulins?

2. Most antibody activity in human serum is due to what three types of antibodies?

3. Antibodies against bacteria endotoxins are primarily what antibody?

4. Antibodies against the mumps virus are what type antibody?

5. On the first contact with a particular antigen, what antibody is produced first?

6. On a second or subsequent contact, what antibody predominates?

7. Each immunoglobulin molecule consists of a combination of two types of chains of amino acids designated as ________ chains and ________ chains.

8. What is the quantity of chains of which the IgM molecule consists?

9. What part of the antibody molecule is the binding site in antigen-antibody reactions?

10. List three types of human globulins.

11. Like antigens, what are the two most common properties shared by immunoglobulins (antibodies)?

12. In ultracentrifugation techniques, how are immunoglobulins separated?

13. What method of quantitative assessment of the immunoglobulins is considered to be the most singly powered analytic procedure?

410. Identify the immunoglobulins in terms of their given functions and characteristics.

Functions of Immunoglobulins. A variety of functions have been given for most of the classes of immunoglobulins, although all are undoubtedly constituents of the immune mechanism.

IgG. IgG immunoglobulin is the most abundant class, comprising about 75 percent of the total serum immunoglobulins. IgG is generally considered to be a good precipitating, complement fixing, and neutralizing antibody but a rather poor agglutinating or lysing antibody. Only IgG molecules are small enough to cross the human placenta. It is usually the antibody involved in the secondary response to infection and immunization, and is most often detected later than IgM in primary infections.

IgM. IgM constitutes only about 5% of the serum immunoglobulins but occupies a very important position in the serodiagnostic laboratory because it is usually the first antibody to be detected after primary infection. It is considered an excellent agglutinating and lysing antibody but reacts weakly by precipitation.

IgA. IgA occupies a unique position among the immunoglobulins in that it is found in body secretions (in special form) in addition to comprising about 20 percent of the serum immunoglobulins. This IgA class of immunoglobulins does not fix complement but does contain antibodies which can be demonstrated or detected in other ways. They include antibodies against diphtheria, *Brucella, Escherichia,* ABO and Rh antigens, some nuclear factors, and other antigens. IgA occurs in large amounts in tears, colostrum, saliva, and internal secretions and may provide protection where other types do not occur.

IgD and IgE. IgD and IgE immunoglobulins are found in minute amounts in the serum and, although demonstrated to have certain important functions in the immune mechanism, have not been well characterized yet.

It is important to remember that antibodies against a given antigen may exist in one, two, or three immunoglobulin classes. Most serologic tests are crude assays of the combined activity of those immunoglobulins which give the kind of reactivity the assay is designed to detect, but generally do not reflect the total antibody picture.

Exercises (410):

Match each immunoglobulin in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. The most abundant class comprising about 75 percent of the total serum immunoglobulins.
___ 2. The only molecules small enough to cross the human placenta.
___ 3. The antibody usually involved in a secondary response to infection or immunization.
___ 4. Found in minute amounts in the serum.
___ 5. Demonstrated to have certain important functions in the immune mechanism, but not well characterized.
___ 6. Usually the first antibody to be detected after a primary infection.
___ 7. Found in body secretions in addition to comprising about 20 percent of the serum immunoglobulins.
___ 8. Considered an excellent agglutinating and lysing antibody.
___ 9. Considered a poor agglutinating and lysing antibody.
___ 10. Does not fix complement and includes antibodies against diphtheria, *Brucella, Escherichia,* ABO and Rh antigens, and some nuclear factors.

Column B

a. IgD.
b. IgA.
c. IgM.
d. IgG.
e. IgE.

411. Point out the cells responsible for antibody synthesis and identify given theories of antibody production.

Site of Antibody Production. Now that we know some of the properties and something about antibody structure, we can direct our attention to the site of antibody production in the body and the cells or tissues involved. Recent studies indicate that lymphoid tissue is the primary source of antibodies. Lymphocytes and plasmocytes are thought to be the cells directly involved in antibody protection. As much as 60 to 70 percent of the total antibody yield may be due to lymphocytes and plasma cells. The remaining antibody protection is probably from cells such as those lining the intestinal tract and large macrophage cells. Since both lymphocytes and plasma cells develop from cells of the *reticuloendothelial system,* we may say that this system produces cells which later produce antibodies. Any cell capable of forming an antibody is called an *immunocompetent cell.*

Theories of Antibody Production. Several theories have been advanced as to how antibodies are produced by cells. The most universal are the template theories. The *direct template* theory holds that when a foreign protein comes into contact with normal globulin as the globulin is being formed, the antigen acts as a mold which causes the newly formed globulin to assume a "mirror image" of the antigen. The antigen is like a rubber stamp—stamping out reverse identical images of itself. The *indirect template* theory states that the presence of antigen within the cell affects the genetic memory of the cell so that the cell produces an altered globulin which is passed on to succeeding generations. The *natural template* theory says that preexisting antibody templates are located in clones of mesenchymal cells. (Clones are an aggregate of cells, all of which are descended from a single parent cell.) The clones react with antigens selectively; that is, they react only with certain antigens. When clones and antigens react, cells in the clone mature as plasma cells which produce an antibody specific for the reacting antigen.

Exercises (411):

1. What two types of cells are extensively involved in antibody synthesis?

2. What is an immunocompetent cell?

3. Which theory of antibody production states that the presence of antigen within the cell affects the genetic memory of the cell so that the cell produces an altered globulin which is passed on to succeeding generations?

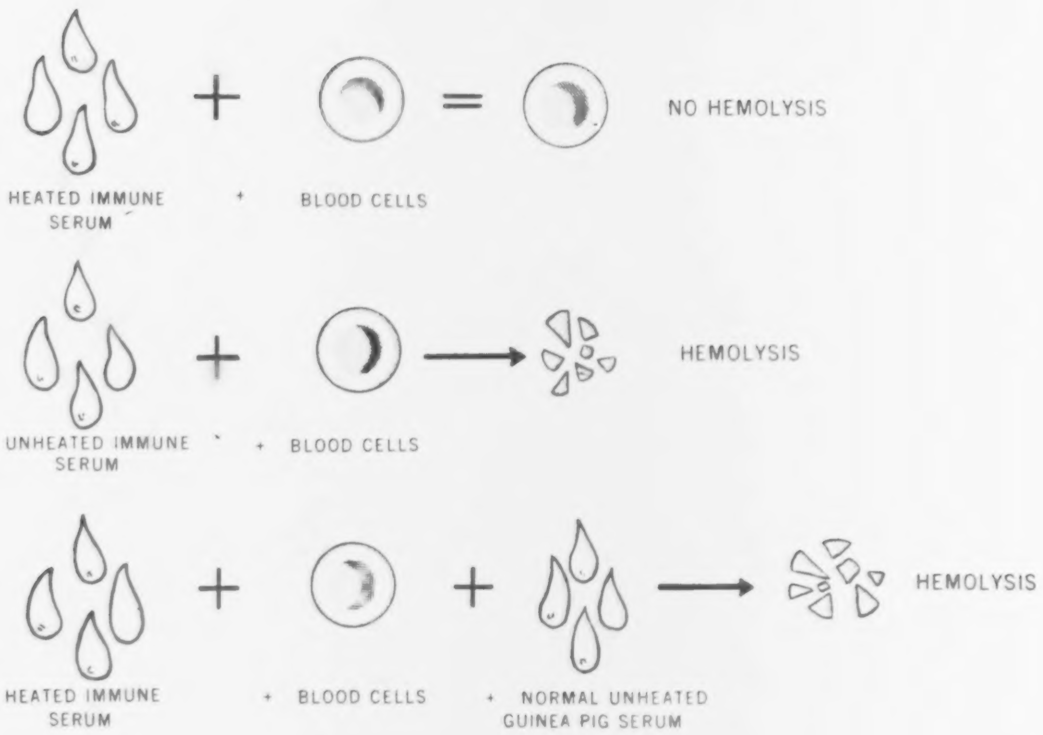


Figure 1-4. Lytic qualities of complement.

4. Which theory of antibody production maintains that there is a "mirror image" mold which produces antibody?

5. What are clones?

412. Indicate whether given statements correctly reflect the definitions, activity, and properties of antibodies.

Complement. No discussion of antigen-antibody interaction is complete without touching upon the role of complement in these reactions. Early bacteriologists noticed that certain bloods would kill pathogenic bacteria when these bloods and bacterial cells were mixed. Only certain kinds of bacteria were destroyed, and the power to destroy was present only in blood of animals immune to that particular type of bacterium. This lytic substance was found in serum as well as in whole blood. This lethal property was destroyed if the blood was heated to about 55° C. for a little less than an hour. The *Pfeiffer reaction* demonstrated that if a test serum was heated to destroy this factor and then mixed with a small amount of normal guinea pig serum, the test serum would again destroy the microorganisms. The conclusion was drawn that the lytic substance was sensitive to heat and that some other heat resistant component (antibodies) in serum is also necessary for the reaction to take place.

Bacteriolysis was not the only type of reaction this factor was responsible for. It was further demonstrated that this same lytic substance (now known as complement) would cause hemolysis with an antiserum prepared against the cells. Cytolysis or cell destruction of both bacteria and blood cells is the direct result of an antigen-antibody reaction in the presence of the lytic factor, complement. The historical observations on the lytic properties of complement are demonstrated in figure 1-4.

Complement occurs in the serum of most animals. The guinea pig is the usual source of complement for testing purposes because guinea pigs yield complement that is more uniform and reliable than the product from most other animals. In order to standardize our serological procedures as much as possible, complement from a single species is necessary.

When antigens and antibodies react in the presence of complement, the complement is actually bound or fixed. It is not available for reaction if other antigens or antibodies are later added to the reaction mixture. This binding effect is called *complement-fixation*. Complement becomes fixed not only in bacteriolytic and hemolytic reactions but other antigen-antibody reactions as well. Some of these reactions do not give macroscopically observable results. Nevertheless, the reaction takes place and may be demonstrated using blood cells sensitized with an appropriate antiserum as an indicator system.

Activity and Properties of Complement. Some of the activities that complement participates in are the following:

- Lethal action against certain bacteria in the presence of an immune serum.
- Lysis of bacteria in the presence of an immune serum.
- Hemolysis of blood cells sensitized with an antiserum.
- Opsonization of certain bacteria, for example, increasing the susceptibility of bacteria to phagocytosis.

Experimentally, complement has been shown to be composed of several distinct globulin and globulinlike components. Individual complement components have been designated with numerals and are termed complement (abbreviated C) "components" or "subcomponents." The numerals in order of their sequence indicate the status of their hemolytic activity. The system is now represented in the following manner. Complement (C) individual components are designated as C1, C2, C3, C4, C5, C6, C7, C8, C9. Component C1 is comprised of three subunits; these are represented as C1q, C1r, C1s.

Complement is now defined as a group of naturally occurring macromolecules that interact with some antigen-antibody complexes in a sequential manner to terminate in irreversible damage to the cell membrane. Complement itself does not possess the ability to lyse non-sensitized cells. At the present time, the group consists of at least nine components, C1 through C9, or eleven serum proteins, each of which has a specific physiochemical and immunochemical property.

Some Additional Properties of Complement. The molecular weights of the complement components range between 79,000 and 400,000. The carbohydrate moiety (equal part or portion) of the components is comparatively large and accounts for 14% of the molecular mass of C4. In one instance, the carbohydrate moiety appears to be essential for the hemolytic activity of the component, C9.

Exercises (412):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. In the early studies of complement, lytic substance was found in serum only.

T F 2. The lethal property found in serum was destroyed if heated to 55° C.

T F 3. Cytolysis of both bacteria and blood cells is the direct result of an antigen-antibody reaction in the presence of the lytic factor, complement.

T F 4. The rabbit is the usual source of complement for testing purposes because rabbits yield complement that is more uniform and relaible than the product from most other animals.

T F 5. When antigens and antibodies react in the presence of complement, the complement is actually bound or fixed.

T F 6. Complement becomes fixed in antigen-antibody reactions but not in bacteriolytic and hemolytic reactions.

T F 7. Reactions that are not observable macroscopically may be demonstrated by using unsensitized blood cells with an appropriate antiserum as an indicator system.

T F 8. Complement increases the susceptibility of bacteria to phagocytosis.

T F 9. Complement is composed of albumin components.

T F 10. The numerals of complement in order of their sequence indicate the status of their hemolytic activity.

T F 11. The antigen-antibody complexes involving complement terminate in damage to the cell membrane that may be reversible.

T F 12. In one instance the carbohydrate moiety appears to be essential for the hemolytic activity of the component C1.

413. State the basic concept of antigen-antibody reaction, cite the forces responsible for this reaction, and identify antigenic sharing and point out its significance.

Immunological Reactions. The science of serology deals primarily with the detection of antibodies present in the body fluids. These antibodies are produced as a specific defense mechanism against certain substances, for example, antigens (immunogenic agents). By demonstrating the presence of a specific antibody *in vitro*, clinical serology supplies indirect evidence of the immunogenic agent which stimulated the antibody formation.

The reaction which occurs between antigen and antibody is only partially understood. It is, however, an extremely important one for the clinical laboratory. We may compare an antigen-antibody reaction-complementary fit to a lock and key concept. This provides a visual example of the primary requisite for an antigen-antibody reaction-complementary fit. The lock (antigen) has tumblers (determinants) that have dimensions. The key (antibody) has grooves (combining sites) that fit exactly into the tumblers of the lock. When the key is inserted and turned, the door opens; when the antigen and antibody combine, a reaction takes place.

Specifically, certain atoms or groups of atoms on the antigen combine with complementary atoms on the antibody. The forces that hold antigen-antibody complex together are: ionic bonds, hydrogen bonds, Van der Waals forces, and hydrophobic bonds. Van der Waals forces result from the mutual polarization of the external electron clouds of the two atoms. The binding forces between antigens and antibodies are generally weak, so the antigen and antibody must be brought close together before reaction occurs. For example, antigenic determinants on a red cell with complementary regions on the antibody combining sites serve to illustrate this point. The complementary regions on the antibody are the antibody sites. Reactions take place at a single site or may involve several sites. In some reactions the molecular binding force may be fairly strong, even irreversible or unalterable. In others it may be weak and reversible.

Antigenic Sharing—Cross-Reactivity. Antigen-antibody reactions involve *specificity*. That is, antigenic determinants ordinarily react with antibody combining sites *only* if they are complementary. In some instances, though, two antigenic determinants are so similar structurally that they react with a common antibody combining site. When this occurs, we speak of it as *cross-reactivity*. Cross-reacting antigens frequently make serological tests difficult to interpret, particularly among those viruses and bacteria which share a common antigen.

Cross-reaction between an antibody and several different antigens occurs mainly among antibodies that are polysaccharide in nature. Many medically important microorganisms have polysaccharide substances as part of their chemical and physiologic makeup. These substances may be either a somatic or capsular antigen. The illustrations in figures 1-5(A) and 1-5(B) demonstrate ways in which cross-reaction may occur.

Significance of Cross-Reactivity. To the serologist, cross-reactivity is both a useful and, at times, a very confusing occurrence. The serologist in performing his tests must be constantly aware of the possibility that some completely unrelated antigen, such as a non-pathogen, may have stimulated the production of the antibodies under study. He must learn to rule out these so-called "false positive" reactions. At times this poses an insurmountable obstacle. For instance, in *Salmonella* typing, a cross-reaction between species would be a handicap to precise identification. However, if we wish to screen, as in febrile agglutination tests, it becomes helpful in reducing the number of tests we have to perform. Thus, on the other hand, the principle of cross-reactivity provides the basis for many useful serological tests. For example, patients with the supposedly viral disease, infectious mononucleosis, produce antibodies against sheep and horse red blood cells. This fact is then used in the laboratory diagnosis of infectious mononucleosis because sheep or horse red blood cells are more readily available and easier to use than the causative virus. In fact, no practical test is available for the infectious mononucleois virus. Another good example is seen with rickettsial diseases, such as typhus and Rocky Mountain spotted fever. These rickettsiae have some antigens in common with certain bacteria of the genus *Proteus*. The cross-reaction between polysaccharide from certain strains of *Proteus* organisms and antibodies against certain *Rickettsiae* is called the Weil-Felix reaction. This cross-reaction is useful in diagnostic tests for rickettsial diseases. Since bacteria are more readily cultivated than rickettsiae, the more economic route is selected by using bacterial antigens *(Proteus sp.)* in serologic testing for the unrelated rickettsial diseases. These are but three examples of the beneficial use of cross-reactivity by the serologist. Many more uses of cross-reactivity are seen in serologic testing, including the most common of serologic tests—the ones for syphilis.

Exercises (413):

1. The "lock and key" concept has been used to describe what feature of antigens and antibodies?

2. What are the forces that hold antigen-antibody complex together?

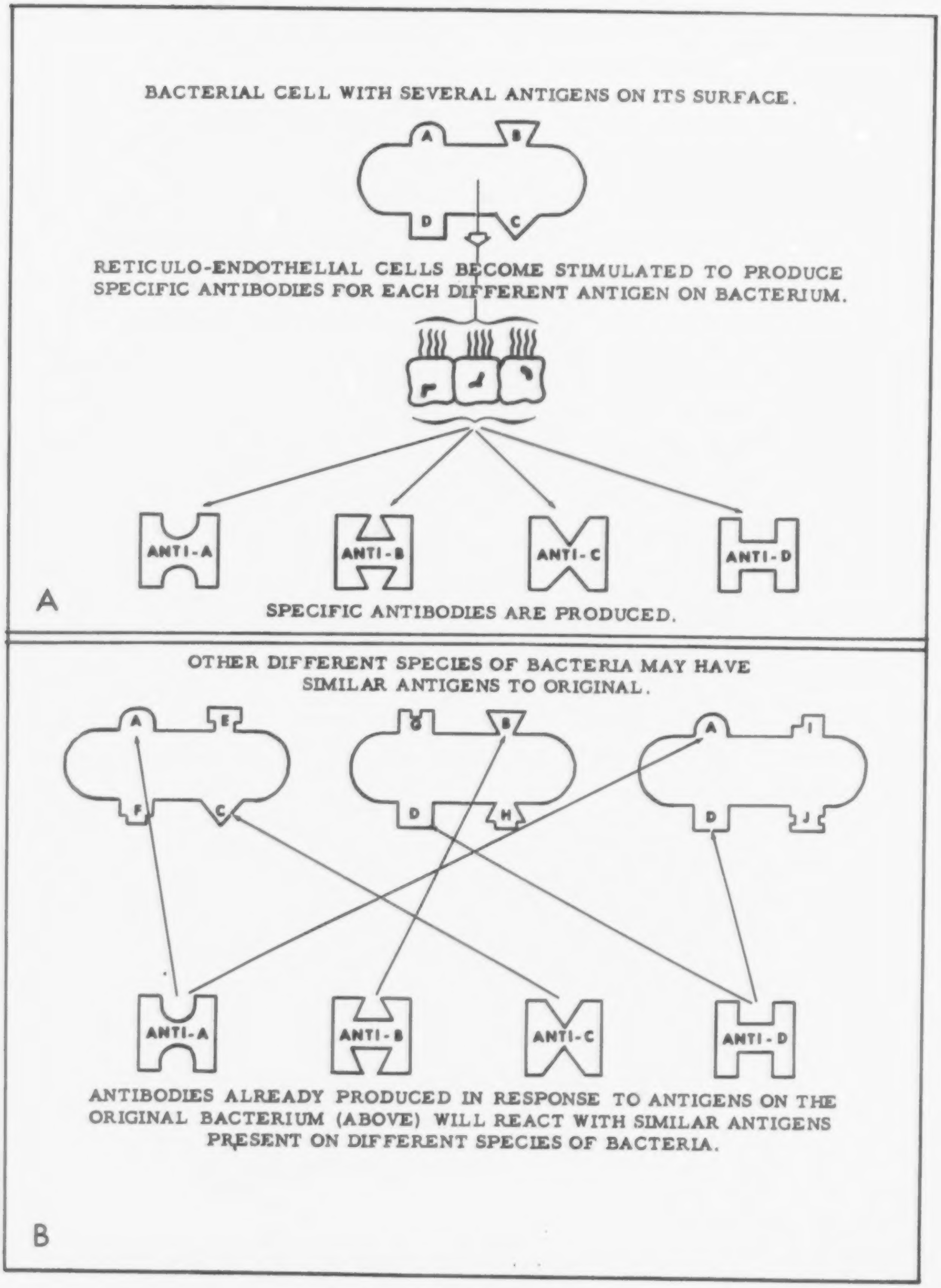
3. Are antigen-antibody reactions reversible? Explain.

4. What kind of reaction does specificity imply?

5. What antigen-antibody reaction occurs in cross-reactivity?

6. Why are cross-reacting antigens and antibodies not always a disadvantage?

7. List three examples in which cross-reacting is beneficially used by the serologist?



BACTERIAL CELL WITH SEVERAL ANTIGENS ON ITS SURFACE.

RETICULO-ENDOTHELIAL CELLS BECOME STIMULATED TO PRODUCE SPECIFIC ANTIBODIES FOR EACH DIFFERENT ANTIGEN ON BACTERIUM.

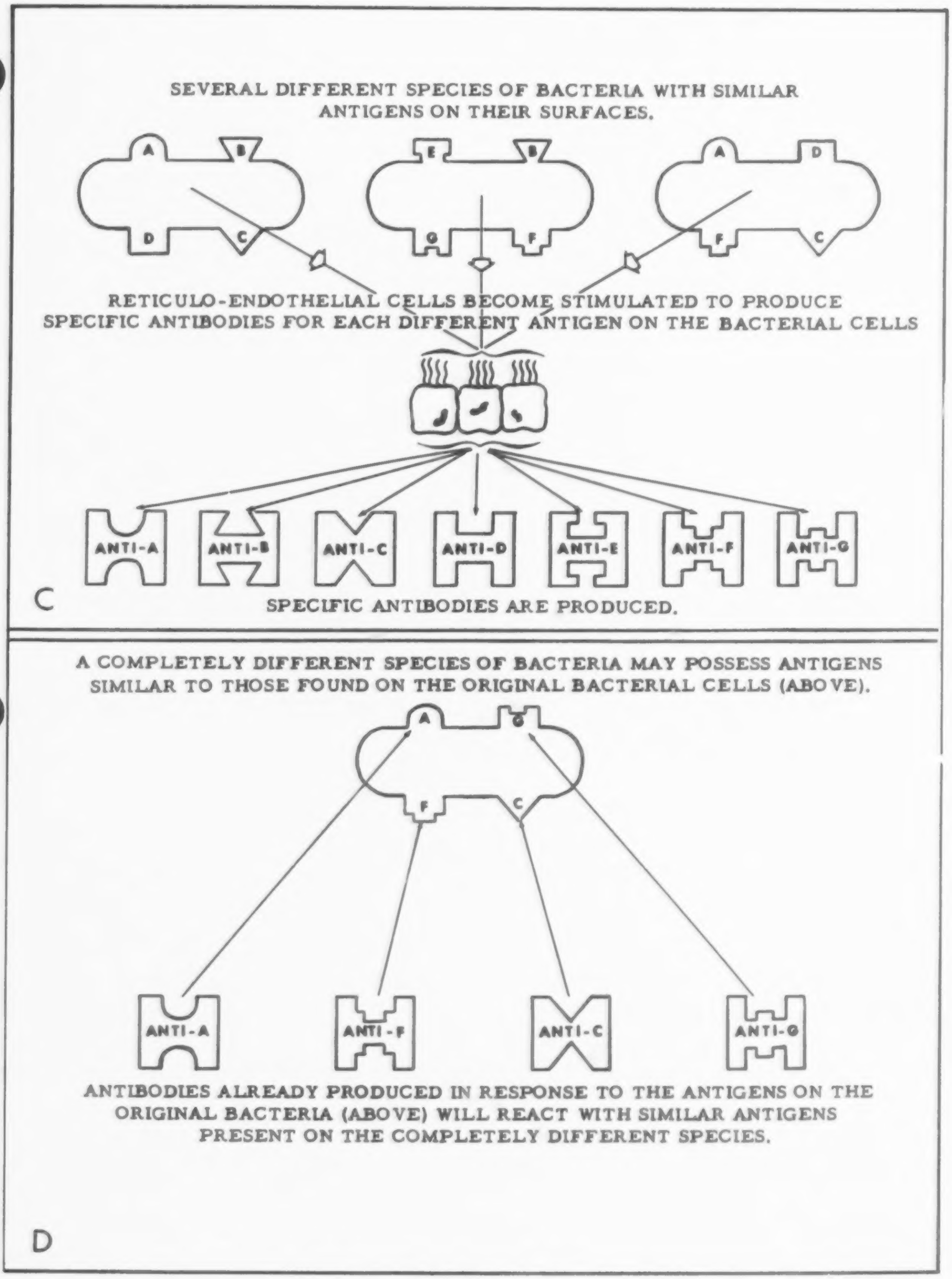
SPECIFIC ANTIBODIES ARE PRODUCED.

OTHER DIFFERENT SPECIES OF BACTERIA MAY HAVE SIMILAR ANTIGENS TO ORIGINAL.

ANTIBODIES ALREADY PRODUCED IN RESPONSE TO ANTIGENS ON THE ORIGINAL BACTERIUM (ABOVE) WILL REACT WITH SIMILAR ANTIGENS PRESENT ON DIFFERENT SPECIES OF BACTERIA.

A
B
C
D
ANTI-A
ANTI-B
ANTI-C
ANTI-D
A
A
E
F
C
G
B
D
H
A
I
D
J
ANTI-A
ANTI-B
ANTI-C
ANTI-D
B

Figure 1-5(A). Cross-reactivity.



SEVERAL DIFFERENT SPECIES OF BACTERIA WITH SIMILAR ANTIGENS ON THEIR SURFACES.

RETICULO-ENDOTHELIAL CELLS BECOME STIMULATED TO PRODUCE SPECIFIC ANTIBODIES FOR EACH DIFFERENT ANTIGEN ON THE BACTERIAL CELLS

SPECIFIC ANTIBODIES ARE PRODUCED.

A COMPLETELY DIFFERENT SPECIES OF BACTERIA MAY POSSESS ANTIGENS SIMILAR TO THOSE FOUND ON THE ORIGINAL BACTERIAL CELLS (ABOVE).

ANTIBODIES ALREADY PRODUCED IN RESPONSE TO THE ANTIGENS ON THE ORIGINAL BACTERIA (ABOVE) WILL REACT WITH SIMILAR ANTIGENS PRESENT ON THE COMPLETELY DIFFERENT SPECIES.

A
B
D
C
E
B
G
F
A
D
F
C
ANTI-A
ANTI-B
ANTI-C
ANTI-D
ANTI-E
ANTI-F
ANTI-G
C
A
G
F
C
ANTI-A
ANTI-F
ANTI-C
ANTI-G
D

Figure 1-5(B). Cross-reactivity. (cont)

414. Indicate whether given statements correctly reflect the causes and results of zonal reactions.

Zonal Reactions. There are several factors, such as ionic concentration, temperature, incubation time, and others, that affect antigen-antibody reactions. However, the serologist rarely has to worry about these factors because most reagents used in serology are commercially manufactured. All the serologist has to do is follow the manufacturer's directions with adequate controls, and these factors will be kept under control. However, there are two situations of which the serologist should be aware. Both may result in a weak reaction or lack of visible manifestation of the expected reaction due to alterations in the optimal proportions of antigen to antibody.

Prozone reactions. Prozone reactions are weak or negative reactions due to an antibody excess. For example, in an agglutination reaction, each antigenic determinant might be combined with a single antibody molecule so that agglutination would be impossible. A prozone reaction is depicted graphically in figure 1-6. Suspected prozone reactions can be detected by making dilutions of the antibodies (serum) before adding the antigen.

Postzone reactions. Postzone reactions are weak or negative reactions due to an excess of antigen in the test. Although rarely encountered by the serologist unless he is devising or modifying a test, postzone reactions are, in fact, what are seen early in a disease before an optimal antibody level is achieved. Some undetectable antibody may be present. A postzone reaction is depicted graphically in figure 1-7.

Exercises (414):

Indicate whether each of the following statemetns is true (T) or false (F) and correct those that are false.

T F 1. Several factors such as ionic concentration, temperature, and incubation time affect antigen-antibody reaction.
T F 2. Only weak reactions will be due to excess antibody.
T F 3. Suspected prozone reactions can be detected by making dilutions of the antigen before adding the antibodies (serum).
T F 4. Postzone reactions are weak or negative reactions due to an excess of antibodies.
T F 5. Postzone reactions are seen early in a disease before an optimal antibody level is achieved.

1-3. Serologic Methods

Serological tests are among the oldest diagnostic tests performed in the laboratory. It has only been recently, however, that we have begun to understand the true nature of microbial and other antigens and their corresponding antibodies. With greater insight, modern serological procedures have provided the immunologist with tools to measure their presence and properties.

415. Indicate whether given statements correctly reflect the definition, general procedures, and purpose of antibody titer.

Titer. Antibody is often measured by making several dilutions of the antibody specimen (usually serum) and allowing it to react with a constant volume of antigen. If the reaction is visible or observable we will see the reaction immediately or within a very few minutes. Sometimes an *indicator system,* usually a cell suspension, must be added to the mixture to make the reaction visible. When reporting results, the expression *titer* is used. Titer is defined as the concentration of antibodies in serum expressed as the reciprocal of the highest dilution giving complete agglutination or

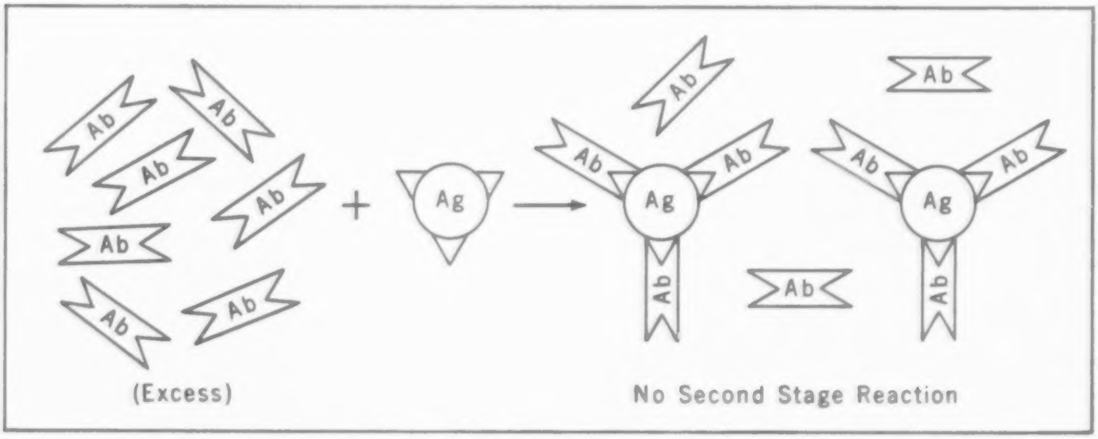


Figure 1-6. Prozone (excess antibody) reaction.

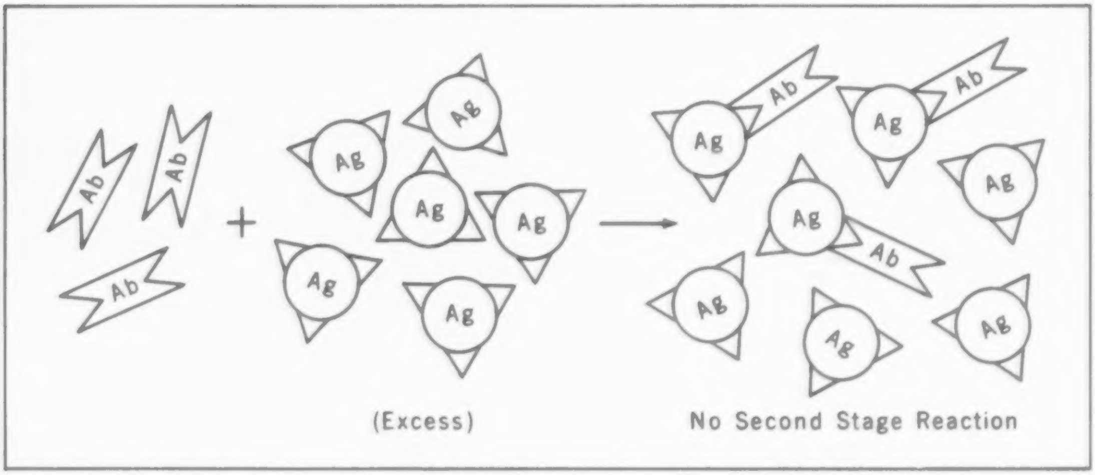


Figure 1-7. Postzone (excess antigen) reaction.

maximum precipitation. Titer expresses the units or parts present in a total volume.

Titers are more accurately performed in tube procedures than in slide procedures. Recent improvements in purified antigens have allowed us to do fairly accurate rapid slide procedures. In fact, in some antigen-antibody mixtures, the reaction is much easier to read on a slide than in a tube (pregnancy test, R-A test). Titering is a simple procedure and is routinely used to quantitate the amount of antibody present in a serum specimen.

Exercises (415):

Indicate whether each of the following statements is true (T) or false (F) and correct those that are false.

T F 1. Antibody is most often measured making one single dilution of the antibody specimen and allowing it to react with a constant volume of antigen.

T F 2. The antibody indicator system is usually similar to a pH indicator system.

T F 3. The concentration of antibodies in serum expressed as the reciprocal of the highest dilution giving complete agglutination or maximum precipitation defines serial dilution.

T F 4. Titers are more accurately performed in slide procedures than tube procedures.

T F 5. Recent improvements in purified antigens have allowed us to do fairly accurate rapid slide procedures.

T F 6. Titer is a simple procedure and is routinely used to quantitate the amount of antibody present in a serum specimen.

416. Cite the sources of cell suspensions and the medium used; calculate cell suspensions from the given formulas and point out sources of error.

Cell Suspensions. Cells from various sources may be used as an antigen source or as an indicator system in serologic tests. The type of cells selected depends on the type of antigen they carry and the type of antibody we want to isolate or quantitate. Cells from humans, cows, sheep, guinea pigs, chickens, horses, and several other animals are routinely used in one serologic test or another. Usually a suspension of these cells is prepared in saline or albumin. Other media may be required, depends on the type of antibody involved.

The mathematics used in calculating cell suspensions is very simple. The following three formulas will solve any cell suspension problem:

(1) To find the packed cell volume when the total volume and amount of cells are known:

$$\text{Packed cell volume} = \frac{\text{total volume X \% solution desired}}{100}$$

(2) To find the percent solution when the total volume and amount of cells are known:

$$\text{\% solution} = \frac{\text{packed cell volume X 100}}{100}$$

(3) To find total volume when final concentration and amount of available cells are known:

$$\text{Total volume} = \frac{\text{packed cell volume X 100}}{\text{\% solution desired}}$$

Be careful in preparing cell suspensions. Don't cut cell washing techniques short or alter them. Skimping in any area leads to false results. Use only fresh cells, because metabolic changes in aged cells may affect test results. Old cells also require more effort in washing because they are easily hemolyzed. (When mixing cell suspensions always use paraffin film or a stopper to cover the tube opening, never your finger.)

Exercises (416):

1. Cell suspensions are obtained from what sources?

2. In what media are the cell suspensions prepared?

3. To make 75 ml. of a 3-percent suspension of sheep blood cells, what volume of packed cells is required?

4. If you have 4.4 ml. of packed cells after washing and the procedure requires that you add a sufficient quantity of saline to make 100 ml. total volume, what percent cell suspension will you have?

5. You have 0.36 ml. of cells after washing and you want to make a 3-percent solution. What volume of cell suspension can you prepare?

6. What are some sources of error in preparing cell suspensions?

417. Define serial dilution; state the formula and calculate the serial dilution using the given formula.

Serial Dilutions. In diluting, a solution of higher concentration is made into one of lesser concentration. If this is done in a mathematical progression, for example, reducing the concentration by half each time, we have a "serially diluted" specimen. When we reduce the concentration by half we are performing a *twofold* serial dilution. In a serial dilution the dilution is inversely proportional to the concentration of the substance being diluted. Consequently, our dilution technique must be standardized so that when we say 1 part serum in a 1 in 10 dilution, everyone will understand the meaning. This statement means we have a total of 10 parts; 1 part is solute and 9 parts are diluent. A 1 in 10 dilution should *not* be interpreted as a 1 part solute to 10 parts diluent, or a total of 11 parts. The dilution may be written as 1/10 or 1:10. If the dilution is a test result expressed as a titer, it can be written as 1/10, 1:10, or "a titer of 10."

You will usually perform serial dilutions in test tubes. However, they can also be performed directly on a ringed slide or a slide with depressions such as a Boerner slide. One of the newer techniques for performing serial dilutions is the microtitration method originally introduced by Takatsy and modified by Sever. This method involves several simple pieces of equipment that may be obtained commercially. The basic components are a Plexiglas sheet and a calibrated loop. The Plexiglas sheet is drilled with a series of wells. U-shaped wells are used for complement-fixation tests and V-shaped cups for hemagglutination tests. Calibrated dropping pipettes are used to deliver the specimen to be tested and other reagents involved. This equipment is shown in figure 1-8.

The correct amount of diluent and specimen are delivered to the appropriate cups with dropping pipettes. The dilution is made by transferring a loopful from one cup to another, mixing, and transferring a loopful. This is a very rapid technique and cuts down tremendously on time and equipment required to perform a large number of serological procedures. The loops are cleaned by flame and the plexiglas plate is easily washed. Repeat tests are new tests can be quickly started and completed. Those laboratories performing complement-fixation, hemagglutination, and similar procedures would so well to consider this technique.

The simplest method of calculating serial dilutions is to use the following formula:

$$\text{Dilution} = \frac{\text{total volume in tube}}{\text{volume of serum in tube}}$$

The answer is always the reciprocal of the dilution. Consider the following problems which demonstrate this formula.

Problem situation #1. You are setting up a test and you need to know the dilution of the specimen that will give maximum results. Start from table 1-2. What is the dilution in the first tube? Continue to calculate the dilutions through tube 7.

Problem situation #2. You have solved the problem down to tube 7 and have obtained the results shown in table 1-3. Calculate the dilution of tube 8.

Problem situation #3. Suppose you wanted to make a one-tube dilution with the following proportions:

Total volume = 7 ml.
Volume serum added = 0.03 ml.

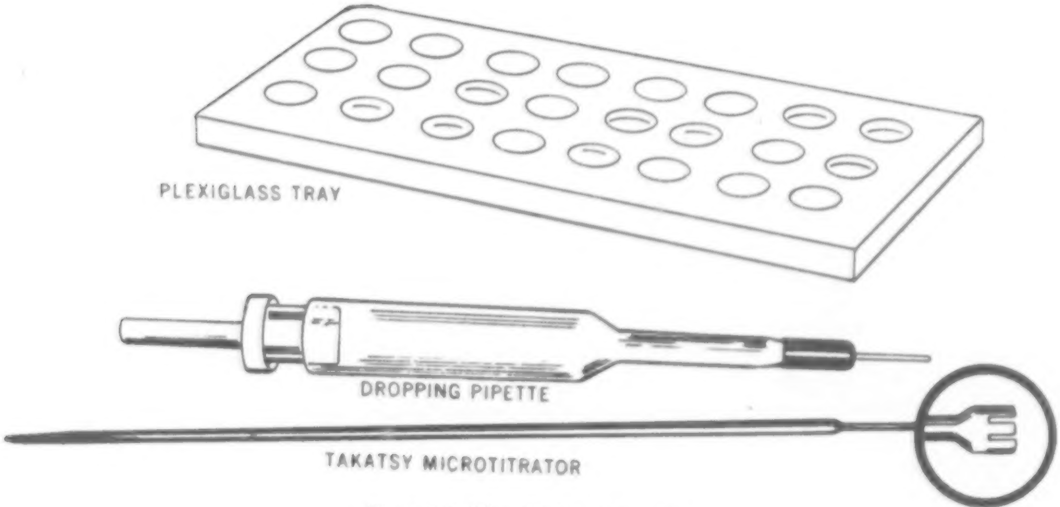


Figure 1-8. Microtitration apparatus.

What is the dilution of the serum?

Solution to problem situation #1. In this instance, you can use the formula directly and solve the problem as follows:

$$\text{Total volume in tube} = (0.5 + 0.5) - 0.5 = 0.5$$

$$\text{Dilution} = \frac{0.5}{0.25} = 2 \text{ or } 1{:}2$$

Solution to problem situation #2. Use the formula to solve the problem as follows:

$$\begin{aligned}\text{Total volume} &= (\text{diluent} + \text{diluted specimen}) \\ &\qquad - \text{transferred volume} \\ &= 0.5 + 0.5 - 0.5\end{aligned}$$

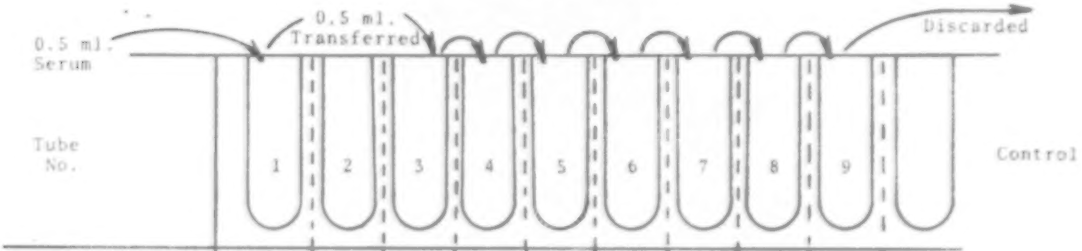
$$\text{Dilution} = \frac{0.5}{0.0019} = 256 \text{ or } 1{:}256$$

Solution to problem situation #3. This problem is solved in a similar manner.

$$\text{Dilution} = \frac{\text{total volume}}{\text{volume serum in tube}} = \frac{7.0}{0.03}$$
$$= 233.33 = 1.233.33$$

Generally, once you find the dilution of the first tube, the others are easy. You should remember that titers or dilutions are sometimes figured differently for different kinds of tests. In immunohematological or blood banking tests the dilution or titer is usually figured prior to adding the cell suspension. On the other hand, in serological procedures the dilution or titer is usually calculated after the cell suspensions,

TABLE 1-2
SERIAL DILUTION SCHEMATIC



Tube No.	1	2	3	4	5	6	7	8	9	Control
Volume Diluent	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vol. Serum After Transfer	0.25									
Dilution										

TABLE 1-3
SERIAL DILUTION RESULTS

	1	2	3	4	5	6	7	8
Vol. Serum after trans	0.25	0.125	0.0625	0.03125	0.01563	0.0078	0.0039	
Dilution	2	4	8	16	32	64	128	

hemolysin, or complement are added. In the first instance the titer is based on the initial dilution of the serum. In the latter, the titer is based on the final dilution in the tube.

Exercises (417):

Complete each sentence with the appropriate word or words where indicated. A phrase rather than a single word may be required for the correct response.

1. What does serial dilution mean?

2. When we reduce the concentration by half we are performing a ______ serial dilution.

3. The statement "a 1 to 20 dilution" means ______ part is solute or specimen and ______ parts diluent or a total of ______ parts.

4. What type of instrument is used in a Takatsy microtitration to make the dilution?

5. What is the formula for calculating serial dilution?

6. What is the dilution of the serum if the total volume is 10 ml. and the volume of serum is 0.025 mls?

7. In blood banking tests, the dilution or titer is usually figured ______ adding the cell suspension, and in serological procedures in the dilution or titer usually calculated ______ the cell suspensions are added.

418. Identify the given serological tests and test reactions in terms of their basic principles, features, and purposes.

Some Common Serological Techniques. The following general methods are used in serology. Some of them will be discussed in later chapters when we consider more specific serological tests.

Complement fixation test. Complement is a set of 11 serum constituents which become inactivated (fixed) in many antigen-antibody reactions. In fact, complement is required for the completion of many of these reactions. However, since many antigen-antibody reactions do not result in a visible manifestation, the detection of complement fixation provides a useful serologic test. To detect whether complement fixation has occurred in the first reaction, a second antigen-antibody system is added to the test. Sheep erythrocytes and antisheep erythrocyte antibodies (hemolysins) are usually used in this second step. The sheep RBC hemolysins require the presence of complement to lyse the erythrocytes. Therefore, lysis of the erythrocytes indicates that free complement is still present in the system or that no complement fixation (no antigen-antibody reaction) occurred in the first step of the test. Conversely, no hemolysis indicates a positive complement fixation test. This test has many applications in the serodiagnosis of viral diseases and has also been used extensively in syphilis serology and is performed at large specialized laboratories.

Agglutination tests. In these tests, reactions are similar to precipitation reactions in that antigen-antibody aggregates are formed. The main difference is that agglutination is used to describe the aggregation of particulate antigens. These antigens might be red blood cells, bacteria, or even inert particles such as latex particles that have been coated with a given antigen. Flocculation is at times used erroneously as a synonym for agglutination. Some authors use agglutination when describing reactions involving cellular antigens such as red blood cells and flocculation for inert antigens such as latex particles. Many varieties of agglutination or flocculation reactions have been applied to clinical serology. They are faster than precipitin reactions and many can be observed macroscopically.

Hemagglutination-inhibition tests. Tests in which the agglutination of cellular antigens such as rbc's are inhibited by exposing them to specific competing antibodies.

Precipitin tests. In these tests the antigen-antibody reaction produces a visible precipitate, a flaky sediment, or soluble antigen-antibody complexes.

Neutralization reactions. Usually neutralization is used only to describe antigen-antibody reactions which require a laboratory animal to detect whether or not a reaction has taken place. The antibodies and antigens, such as toxins or viruses, are mixed in vitro. Then the mixture is injected into the test animal. Subsequent observation of the animal for characteristic symptoms indicates whether the toxin or virus has been neutralized. As can be imagined, the maintenance of an animal colony is beyond the scope of the average clinical laboratory. However, if one uses the term "neutralization" in a broader sense, there are several serologic tests that could be categorized as neutralization reactions. For example, the hemagglutination inhibition tests previously discussed might apply, but the best example is the antistreptolysin-O (ASO) test. The antigen in this test is a hemolysin, streptolysin-O, that is produced by certain streptococci. Antibodies neutralize the hemolysin in vitro so that when erythrocytes are eventually added to the test, the hemolysin can no longer hemolyze them.

Immunodiffusion tests. In general, these tests are based on the migration of the antigen and/or antibody through the agar until optimum proportions of each are reacted and a precipitate appears. As usual, the appearance of the precipitate takes several hours, but a modification by combining diffusion with electrophoresis has speeded the reaction and has great promise for the future in serologic diagnosis. When this is done, specific globulin fractions involved in the reaction can be separated.

Exercises (418):

Match each serological test or reaction in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. Sheep erythrocytes and antibodies (hemolysins) are usually used in the second step of this test.
___ 2. This test has many applications in the serodiagnosis of viral diseases and has also been used extensively in syphilis serology.
___ 3. Antigens in this test might be red blood cells, bacteria, or even inert particles such as latex particles.
___ 4. Test in which the agglutination of cellular antigens, such as rbc's, is inhibited by exposing the antigen to specific competing antibodies.
___ 5. A flaky sediment, or soluble antigen-antibody complexes are produced in this test.
___ 6. The best example of this type is the antistreptolysin-O test.
___ 7. These tests are based on the migration of the antigen and/or antibody through the agar until optimum proportions are reacted and a precipitate appears.
___ 8. Specific globulin fractions involved in the reaction can be separated.

Column B

a. Hemagglutination-inhibition test.
b. Immunodiffusion test.
c. Complement fixation test.
d. Agglutination test.
e. Neutralization reaction.
f. Precipitin test.

419. Cite the principle of immunofluorescence reactions, the two types of techniques, and their uses.

Immunofluorescence Reactions. Also called fluorescent antibody (FA) reactions, these tests are based on the fact that certain chemicals emit visible light when exposed to ultraviolet (UV) light. Some of these chemicals (for example, the dye fluorescein isothiocyanate) can be conjugated with antibodies without destroying antibody reactivity. Although there are several modifications, two main types of reactions are used in the fluorescent antibody tests in use in clinical serology.

Direct fluorescent antibody technique. In this method, a specific antibody is conjugated with fluorescein. The labeled antibody is reacted on a slide with its specific antigen, such as bacteria, spirochetes, viruses, or similar particulate antigens. The specific antibodies adhere to the cells. After washing away excess antibody (and only other antibodies not specific for the antigen), the preparation is exposed to UV light and viewed with a microscope fitted with special filters. In the case of fluorescein isothiocyanate-labeled antibodies, the organisms will now emit a blue-green light. This technique is used mainly to detect an antigen in tissue or from culture by using known, labeled antibodies. It has been applied to the detection of the gonococcus, meningococcus, streptococci, plague bacilli, and several others in clinical specimens. The principle of direct staining is shown in figure 1-9. The method is not used for the detection of L. E. factor because it is less sensitive than the indirect method and would necessitate the tedious process of conjugation of sera of individual patients.

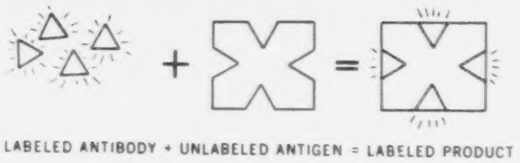


Figure 1-9. Schematic of direct staining with fluorescent antibody.

Indirect fluorescent antibody technique. This modification of the fluorescent antibody technique is useful because it permits the detection of antibody in a patient's serum without the time-consuming process of having to label the antibodies of each patient with the fluorescein dye. While the direct method is similar to the direct Coombs test, the indirect FA method is similar to the indirect Coombs test. As usually applied, the unlabeled patient's serum is reacted with a known antigen (bacteria, spirochetes) on a slide and the excess antibody washed away as in the direct technique. Next, fluorescein-labeled antihuman globulin, which has been produced in an animal following the injection of human globulin, is added. The labeled antihuman globulin reacts with the patient's antibodies (globulins) that have coated the antigens. When examined by UV microscopy, the same fluorescence as in the direct test is seen. The steps in the reaction are diagrammed in figure 1-10. In addition, this test can be performed on serial dilutions of the patient's serum in order to determine the antibody titer. The Fluorescent Treponemal Antibody Absorption (FTA-ABS) test for syphilis and the Antinuclear Antibody Test for lupus erythematosus are based on this indirect FA method.

Exercises (419):

1. What is the basic principle of tests utilizing the fluorescent antibody reactions?

2. What dye is conjugated with the antibodies without destroying antibody reactivity?

3. Which of the two fluorescent antibody techniques is used mainly to detect an antigen in tissue or from culture by using known labeled antibodies?

4. In the indirect fluorescent antibody technique, prior to examination by UV microscopy, what substance is added after the excess antibody has been washed off the known antigen on the slide?

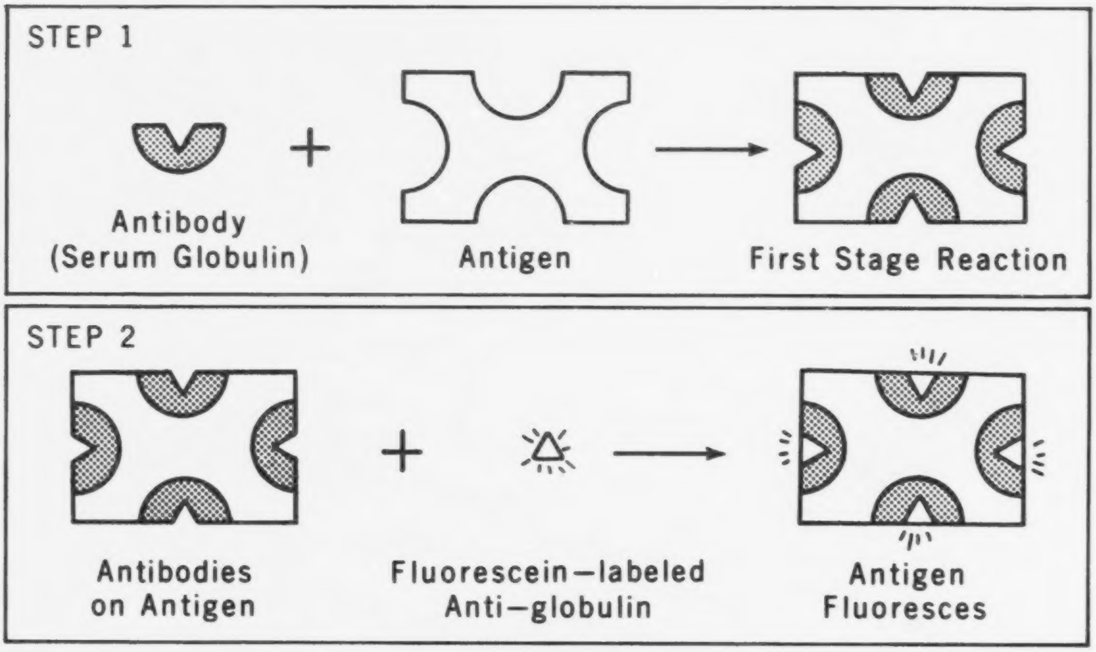


Figure 1-10. Indirect fluorescent antibody.

5. What two tests are based on the indirect FA method?

6. Why is the direct method unsuitable for detection of L. E. factor?

CHAPTER 2

Agglutination Tests

WHEN WE WATCH blood cells agglutinate, we are observing a highly complicated phenomenon. If bacteria are agglutinated by mixing a drop of a broth culture with a specific antiserum, do you suppose the agglutination you observe is any simpler? Very unlikely. In fact the process is just as complicated as when blood cells clump.

How many intermediate steps do you suppose are involved in this process? As you can imagine, very many intricate and complex processes are occurring. In some instances the reactions are immediate and simultaneous, whereas in others certain reactions occur only after the completion of prior reactions. Also, consider special requirements, such as critical temperature, electrolytic media, and optimum concentration of the antigen and antibody.

In the serology laboratory wide use is made of the agglutination phenomenon. Many diagnostic tests are based on this process. This chapter discusses some of the tests in which the agglutination reaction aids in deciding whether or not the patient has been exposed to certain infectious diseases. We shall introduce you to these tests by presenting a few *classical procedures* that will help you understand the nature of the antigens and antibodies which give rise to agglutination. We shall also indicate precautions to be taken to insure properly controlled tests. Where possible, new methods and techniques will be discussed, their components analyzed, and their applicability assessed.

2-1. Serological Tests for Infectious Mononucleosis

Infectious mononucleosis (IM) is a disease frequently tested for in the serology laboratory. It is an acute or subacute benign disease of the reticuloendothelial tissues, especially of the lymphatic tissues. The disease is in some way associated with the *Epstein-Barr (EB) virus* of Burkitt's lymphoma (or some herpes-like virus). Infectious mononucleosis is characterized by fever, headache, sore throat, enlarged lymph nodes, splenomegaly, malaise, and mild hepatitis. Transmission most likely is by intimate oral contact, even though epidemiological observations indicate that kissing is by no means the only means of transmission. Young adults (16-25 years old) are most frequently affected; consequently, infectious mononucleosis is a significant problem for the military services.

Generally, this disease causes the patient to develop a fever and an increased white blood cell count with atypical lymphocytes. During the course of the disease the patient usually exhibits adenopathy (swelling of lymph nodes); splenomegaly (enlargement of the spleen) and occasionally splenohopatomegaly (both spleen and liver enlargement). Although the terms describe some of the symptoms that may beset the patient, they do not always occur. Some people have the disease in a mild form with few of the described symptoms but undergo a strong antibody response, as evidenced by high serological titers. Other persons may have the symptoms in their severest form with little or no antibody response. As you can see, situations such as these tend to be confusing. One writer went so far as to describe the disease as a kind of controlled leukemia. This is certainly understandable when we examine a blood smear from a patient with the disease and see, typically, a wide assortment of definitely abnormal lymphocytes.

In discussing infectious mononucleosis, we shall emphasize several aspects of interest to the serologist. First we will discuss heterophile and other related antibodies. Then we shall examine present thinking concerning the cause of this disease. We will also review the classical or heretofore standard tests for diagnosing the disease. Finally, we will summarize the new slide tests for IM.

420. Distinguish between heterophile and isophile antibodies and cite the three most common heterophile antibodies.

Heterophile Antibodies. We may divide antibodies into two broad categories based on the source of the antigen that caused their production. If an antibody reacts with antigen from *all* members of a certain species of animal such as all sheep, all humans, or all horses, but reacts *only* with antigen from that certain species, then it is an *isophile antibody*. Antibodies that react with antigens from several different species of organisms are *heterophile antibodies*. The word heterophile is derived from the Greek words *heteros*, meaning other, and *philein*, meaning to love. They may be thought of as antibodies which will react with antigens other than the specific antigen that caused them to be produced. Antigens capable of stimulating the production of heterophile antibodies are called heterophile antigens.

Isophile Antibodies. Isophile antibodies do not occur naturally. They can be produced by injecting suitable antigens into an animal. If a rabbit is given an injection of sheep cells, it will develop several different kinds of antibodies. Some of these will be specific for cell wall nuclear remnants and other cell proteins. A small number of these antibodies will be exclusively specific for sheep cells. These anti-sheep cell antibodies are isophile antibodies and as such will react with all sheep cells. Some of the other antibodies produced by the rabbit will be heterophile antibodies. These antibodies will react with antigens from several sources.

Heterophile antigens are present in a variety of animals, plants, and bacteria. The plant and bacterial heterophile antigens get into the body through ingestion, inhalation, or infection in which they are a component of microorganisms. An immune response to these antigens produces the naturally occurring heterophile antibodies called Forssman antibodies. On the other hand, another type of heterophile antibody is sometimes produced as a result of receiving an injection of horse serum such as in antitoxin treatment of diphtheria. Antibodies produced in this fashion are called serum sickness antibodies. Infectious mononucleosis stimulates the production of yet another heterophile antibody.

Just as the ABO system is only one of the human blood groups, several types of heterophile antigens exist. Heterophile antibodies are similar in that they all react with common antigens in certain conditions, but they also differ very much. In some of these systems the antibodies are hemolysins and hemolyze heterophile antigen-carrying cells. In others, the antibodies are hemagglutinins and agglutinate the heterophile antigen carrying cell. Sometimes, it is a bacterial agglutinin or a precipitin which reacts with certain lipopolysaccharides.

Exercises (420):

1. Distinguish isophile antibodies from heterophile antibodies.

2. When heterophile antigens present in a variety of animals, plants, and bacteria get into the body through injection and inhalation, they produce antibodies known as ________ ________ .

3. Heterophile antibodies produced by the body as a result of receiving an injection of horse serum, such as in antitoxin treatment for diphteria, are called ________ ________ ________ .

4. Heterophile antibodies which are stimulated by infectious mononucleosis are called ________ ________ .

421. Indicate whether given statements correctly reflect the characteristics and features of the given heterophile antibodies.

Significance of Heterophile Antibodies. The three best known heterophile systems are those of Forssman, serum sickness, and infectious mononucleosis. Regardless of the route of entry of the heterophile antigens, all stimulate the production of sheep erythrocyte agglutinins (heterophile antibodies). Fortunately, despite the multitude of heterophile antigens to which man is exposed, heterophile antibodies can be conveniently differentiated by absorption studies and other methods into the three main groups. Let's briefly discuss some characteristics of the three groups.

Forssman (native) antibodies. Forssman discovered in 1911 that rabbits injected with emulsions of guinea pig kidney, adrenals, liver, testes, and brain produced antibodies that hemolyzed sheep cells in the presence of complement and agglutinated sheep cells in the absence of complement. This antigen became known as the Forssman antigen and the antibody it produced as the Forssman antibody. Guinea pig red blood cells and serum did not contain the antigen. Further work showed that the Forssman antigen occurred, inherently, in a variety of organisms. It is found in human Group A and B cells and sheep erythrocytes. It is also found in the horse, dog, cat, mouse, fowl, tortoise, and certain other animals. It is absent in both red blood cells and organs of rabbits, cows, pigs, and rats. Microorganisms possessing the antigen include certain strains of *Salmonella, Francisella, Shigella, Streptococcus,* and *Bacillus.*

Serum sickeness antibodies. Serum sickness antibodies are produced when humans are given an injection containing horse serum. This type of heterophile antibody will rarely be encountered today due to the declining use of horse serum injections. If present, these antibodies react with sheep cells to produce hemagglutination. Since the horse is a source of Forssman antigen you could easily assume that the antibodies in serum sickness were of the Forssman type. This is not so. Serum sickness antibodies differ from Forssman antibodies in several qualities, as you shall see when we discuss serological tests for heterophile antibodies. An injection of horse serum produces antibodies in very much higher titer than is normally found with Forssman antibodies. This property, plus other differences of serum sickness antibodies, indicates that horse serum contains more than one type of heterophile antigen. A patient with serum sickness might, therefore, possess Forssman antibodies in a relatively low titer and serum sickness antibodies of a relatively higher titer.

Infectious mononucleosis antibodies. The infectious mononucleosis type of antibody is also classed as heterophile because it reacts with certain heterophile antigens. We can detect this antibody using the traditional sheep cell hemagglutination test. An anti-sheep agglutinin as well as an anti-sheep hemolysin is present in the serum of most patients having infectious mononucleosis. The hemolysin is not observed in the sheep cell hemagglutination test because the hemolysin requires complement to react. Inactivation of complement in the test specimen keeps the test from showing a hemolytic reaction.

Exercises (421):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. Regardless of the route of entry, all heterophile antigens stimulate the production of sheep agglutinins except for the Forssman antigen.

T F 2. Forssman antigen is found in both red cells and organs of rabbits, cows, pigs, and rats.

T F 3. Forssman antigen is absent in horse, dog, cat, mouse, fowl and tortoise.

T F 4. Microorganisms possessing the Forssman antigen include certain strains of *Salmonella, Francisella, Shigella, Streptococcus,* and *Bacillus.*

T F 5. Like Forssman antibodies, those of serum sickness are produced in response to the type of antigen found in certain strains of microorganisms such as *Salmonella, Shigella* and *Streptococcus.*

T F 6. An injection of horse serum produces antibodies in very much higher titer than is normally found with Forssman antibodies.

T F 7. Anti-sheep agglutinin as well as anti-sheep hemolysin is present in the serum of most patients having infectious mononucleosis.

T F 8. The hemolysin is not observed in the sheep cell hemagglutination test because the hemolysin requires complement to react.

T F 9. Inactivation of complement in the test specimen enables the test to show a hemolytic reaction.

422. Indicate whether given statements correctly reflect some characteristics and the causative agent of infectious mononucleosis.

Etiology of Infectious Mononucleosis. Researchers have searched diligently for the agent or agents causing infectious mononucleosis. Many possibilities have been considered. Most have been discarded as untenable. Bacterial agents were easily eliminated. No usual increase in bacterial organisms is noted even in the most severe forms of this disease. A virus of some kind was considered the most likely cause by most workers. The fact that most viral infections produce adenopathy and atypical lymphocytes correlates very well with conditions found in infectious mononucleosis. Several microorganisms, including *Listeria monocytogenes,* have been isolated from some cases of IM, but not all cases. The observation that lymphocytes produce an IgM immunoprotein is further implication of a virus as the agent.

The work by Epstein and Barr of London in 1964 led to findings of major significance in pinning down the virus responsible for IM. Their work with tissue cultures from cell lines of Burkitt's East African malignant lymphoma led to the discovery of a herpes-like virus within successive generations of lymphoblast cells. The lymphoma cell line was designated Epstein-Barr (EB) and the herpes-like virus was designated Epstein-Barr virus (EBV). Further studies by other workers concerning the serological specificity of the EB virus have shown that it, or a closely related one, is widespread throughout the world.

Recently, striking evidence has been obtained that the Epstein-Barr (EB) virus is the cause of infectious mononucleosis (IM). The relationship of EB virus to infectious mononucleosis is indicated as thus: antibody to EB virus is consistently present in heterophile antibodies positive for IM. In a study of 268 college students, who originally did not have serum EB virus, 15 percent developed typical IM during college and all 15 percent simultaneously developed antibody to EB virus in their sera. A total of 94 students who had a low titer of antibody to EBV did not develop IM. Approximatley 10 percent of adults and even more pediatric patients with IM have no heterophile antibody, but most have antibody to the EB virus. The EB virus has now been isolated from the throats of patients with IM.

Test for EB Virus. The FA test for human antibodies to EB virus has been found to be sensitive, reproducible, and specific. However, the EB virus antibody is distinctive from the heterophile antibody in that it cannot be absorbed out by sheep or beef red cells and it persists for a longer period of time than do the heterophile antibodies. Due to the persistent FA activity of the EB virus FA test, it appears that this test would be of limited value as a routine diagnostic procedure.

Exercises (422):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. The organism *Listeria monocytogenes* had been isolated from all cases of IM.

T F 2. The observation that lymphocytes produce an IgM immunoprotein is further implication of a rickettsia as the causative agent.

T F 3. The work by Epstein and Barr of London led to findings pinning down the rickettsia as responsible for IM.

T F 4. Striking evidence has been obtained that the EB rickettsia is the cause of IM.

T F 5. About 10 percent of adults and even more pediatric patienst with IM have no heterophile titer, but most have antibody to the EB virus.

T F 6. The EB virus has now been isolated from the throats of patients with IM.

T F 7. The FA test for human antibodies to EB virus has been found to lack sensitivity, reproducibility, and specificity.

T F 8. The EB virus antibody is distinctive from the heterophile antibody in that it cannot be absorbed out by sheep or beef red cells, and it persists for a longer time than do the heterophile antibodies.

423. Cite the basis upon which Paul and Bunnell were led to devise the presumptive heterophile test and specify the procedures for the test in terms of their significance.

Paul-Bunnell Presumptive Heterophile Test. The classical sheep cell test for heterophile antibodies was developed from studies begun in 1929 by Davidsohn and in 1932 by Paul and Bunnell. Dr. Davidsohn's study showed that serum sickness produced anti-sheep cell antibodies in a high titer. He further found that these antibodies could be partially absorbed by exposing them to guinea pig kidney and completely absorbed by beef erythrocytes. Paul and Bunnell found that the heterophile antibodies of infectious mononucleosis were not removed by guinea pig kidney but were completely absorbed by beef erythrocytes. They also observed that infectious mononucleosis caused the production fo antibodies in higher titer than either those of Forssman or those caused by serum sickness. This led them to devise a presumptive test for infectious mononucleosis using a 2-percent suspension of sheep cells—a source of antigen.

In the presumptive test you first heat a serum specimen to inactivate complement; if complement is not inactivated, it permits sheep hemolysin to react and causes hemolysis that might give misleading test results. After inactivation of complement (56° C for 30 minutes), serially dilute the serum specimen and add a 2-percent suspension of fresh sheep cells in saline. The sheep cells act both as a source of heterophile antigen and as an indicator of reaction. Allow the tubes to stand at room temperature (20° C) for 2 hours. Read the tubes for visible agglutination; the titer of antibody present is the reciprocal of the last tube showing agglutination. A titer of 56 in the absence of other findings such as atypical lymphocytes and clinical symptoms is insiginificant, and for all practical purposes the specimen is considered negative for IM antibodies. If other findings are present and a titer of 224 or higher is found, the presumptive test is considered positive.

Highest titers are usually found during the second to fourth week of infection. Some people, severely ill with IM, have low or moderate titers; while others, only slightly affected, have very high titers. In some instances persons with a negative or low titer specimen still show both atypical lymphocytes and the usual clinical signs of the infection. Infectious mononucleosis produces anti-sheep cell antibodies in only 50 to 80 percent of those persons infected. Very young children may have very little antibody response. Therefore, in cases exhibiting typical signs but showing a titer of less than 224, a differential heterophile test is required. For example, a titer of 28 or 56 could be due to natural Forssman, serum sickness, or IM antibodies.

Exercises (423):

1. What reaction did Paul and Bunnell observe between heterophile antibodies for IM and guinea pig kidney?

2. What reaction was further observed between IM antibodies and beef erythrocytes?

3. In terms of titer comparison between those antibodies of Forssman, serum sickness, and IM, what further observation led these investigators to devise a presumptive heterophile test?

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4. In the presumptive heterophile, why is the serum heated first?

5. What will result if complement is not inactivated?

6. What purpose does the 2-percent suspension of fresh sheep cell serve?

7. What is the result of the test if a titer of 56 is obtained in the absence of other findings such as atypical lymphocytes and clinical symptoms?

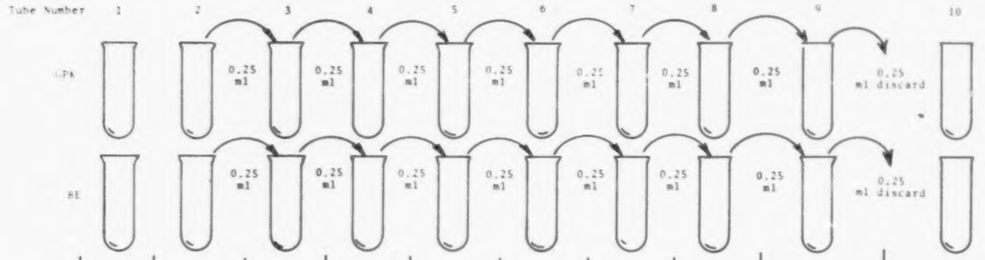
8. In cases exhibiting typical signs but showing a titer less than 224, what further test should be done?

424. State the characteristic reactions obtained from the Davidsohn differential test with the three heterophile antibodies; cite procedures of the test and a source of error.

Davidsohn Differential Test. A test to differentiate the three common types of heterophile antibodies was devised by Dr. Davidsohn as a continuation of his work with the serum sickness antibodies that he reported upon in 1929. As mentioned before, he found that serum sickness antibodies were partially absorbed by guinea pig kidney (GPK) and completely removed by beef erythrocytes (BE). In the differential test, you inactivate the patient's serum complement as before. Then mix an aliquot of test serum with a suspension of macerated guinea pig kidney, and mix another aliquot with a suspension of boiled beef erythrocytes. Allow both mixtures to stand at room temperature for 3 to 6 minutes; then spin the mixtures at 1500 rpm for 10 minutes or until the supernatant is clear. Remove the supernatant from each to a separate clean test tube. Only the IM antibodies are left unabsorbed in the supernatant solution. Remember that in the process of absorbing the unwanted Forssman and serum sickness antibodies with GPK and beef erythrocytes, the original serum specimen becomes diluted.

Test aliquots of the supernatant solutions against sheep cells for agglutination in a modified form of the presumptive test shown in table 2-1. Table 2-2 shows the absorption pattern you can expect. Results are usually reported as a titer of each type of absorption, such as GPK 1:112 BE 1:7. In positive tests the GPK supernatant solution differs by no more than a three-tube drop in titer as compared to the presumptive test.

TABLE 2-1
DAVIDSOHN DIFFERENTIAL HETEROPHILE TEST



Tube Number	1	2	3	4	5	6	7	8	9	10
Saline ml	None	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Supernatant Fluid (1:5 dilution of serum)	0.25	0.25	---	---	---	---	---	---	---	---
Serum Transfer ml	---	---	0.25 milliliters of							None (Control)
			1:10	1:20	1:40	1:80	1:160	1:320	1:640	
Dilution After Serum Transfer	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	---
Sheep Corpuscles ml	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total Volume ml	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Final Dilution of Serum	1:7	1:14	1:28	1:56	1:112	1:224	1:448	1:896	1:1792	---

Mix thoroughly and keep at room temperature for 2 hours; make readings.

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TABLE 2-2
ABSORPTION PATTERNS OF HETEROPHILE ANTIBODIES

Type Antibody	GPK	BE
Serum Sickness	Absorbed	Absorbed
Forssman	Absorbed	Not Absorbed or partial
Infectious Mononucleosis	Not Absorbed or slightly	Absorbed

The BE supernatant solution will give a very low or negative result in tests positive for IM. Table 2-3 gives a random sampling of a few possible combinations of GPK and BE test results. Other combinations are possible. You will rarely encounter presumptive tests of 1:224 or higher that yield a negative IM differential test pattern. Misleading test results may be due to incomplete inactivation of complement in the specimen.

Exercises (424):

1. In the Davidsohn's differential test, what two suspensions are used to absorb heterophile antibodies?

2. What heterophile antibodies are not or slightly absorbed by guinea pig kidney suspension?

3. What two heterophile antibodies are absorbed by boiled beef erythrocytes?

TABLE 2-3
DIFFERENTIAL TEST INTERPRETATION

Presumptive test titer	Differential test after adsorption with ----		Interpretation with regard to infectious mononucleosis
	Guinea pig Kidney	Beef red cells	
1:224	1:112	1:7	POSITIVE
1:224	1:56	0	POSITIVE
1:224	1:28	0	POSITIVE
1:224	1:14	1:112	NEGATIVE
1:224	1:7	1:112	NEGATIVE
1:56	1:56	0	POSITIVE
1:56	1:28	0	POSITIVE
1:56	1:14	0	POSITIVE
1:56	1:7	0	POSITIVE
1:28	1:28	0	POSITIVE
1:28	1:14	0	POSITIVE

4. In a positive test, how should the GPK supernatant solution differ from the presumptive titer?

5. What could lead to misleading test results when preparing specimen for the differential heterophile test?

425. Indicate whether given statements correctly reflect the methods, principles, and reagents used in other tests for infectious mononucleosis.

Other Tests for IM. Various new tests have been devised to test for the presence of heterophile antibodies. These tests are generally one-step or two-step rapid slide tests. Before discussing some of the more popular ones, we should point out that serological tests are invalid without proper controls. Several other tests for infectious mononucleosis are in use. Most of these are modifications of the basic Paul-Bunnell presumptive and the Davidsohn differential tests; a few are based on different principles. The principle of several of these tests will be described briefly and one or two examples given when commercial products are available. In all cases, be sure to adhere to the manufacturer's instructions in performing these tests.

Ox hemolysin test. In this test ox (beef) erythrocytes and complement are added to serially diluted serum. Patients with infectious mononucleosis produce higher titers of ox hemolysins than normal individuals. Various modifications of this test are in use, but titers of more than 1:40 to 1:48 are usually considered indicative of infectious mononucleosis. This test has been found to be quite specific and sensitive, but time-consuming.

Tests with papain-treated sheep erythrocytes. The proteolytic enzyme, papain, has been found to destroy the receptor sites for infectious mononucleosis antibodies on sheep erythrocytes. Serum is tested against both papainized and untreated sheep cells. In infectious mononucleosis, the untreated cells agglutinate, but the papainized cells do not, or else their agglutination is delayed when compared to the untreated cells. Examples of commercial procedures using this principle are the Bacto-Hetrol Slide Test (Difco) and MonoStat (COLAB).

Modified presumptive tests using horse erythrocytes. Horse erythrocytes have been found to be more sensitive with fewer false positives than sheep erythrocytes for the detection of infectious mononucleosis antibodies. Both formalinized cells and cells preserved in 3.8 percent citrate give acceptable results. Several concentrations of cells have been tested. In addition, when properly stored, these cells are stable for up to 1 year. Examples of slide test using formalinized horse erythrocytes are the IM Kit (Microbiological Research Corporation) and Mono-Test (Wampole).

Modified Davidsohn differential tests. A number of slide modifications of the basic GPK and BE absorption test are available commercially. Since absorption tests seem to be the most widely accepted method for the differential identification of heterophile antibodies, these slide tests offer a distinct advantage—speed—over the original tube test. In addition, these tests delete the need for a presumptive test. An example of a slide differential heterophile test that uses the traditional sheep cells as the indicator antigen is the Monosticon (Organon, Inc) test. Another test kit from Organon, the Monosticon Dri-Dot, consists of separate spots of dried GPK and horse erythrocytes on a paper card. The serum is added to the GPK. Then, this mixture is stirred into the horse erythrocytes. Another test kit that uses horse erythrocytes, but in suspension form, along with both GPK and BE suspensions is the Monospot Slide Test (Ortho).

An important consideration in using any of these tests is to be sure to follow the manufacturer's instructions at all times. Although some of these manufacturers include procedures for titrating the antibodies, titrations would not appear to be economically feasible in view of the fact that the titer does not parallel the severity of the disease. Liver function tests are considered to be better than titrations for following the course of the disease.

Exercises (425):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. In the ox hemolysin test, beef erythrocytes and guinea pig kidney are added to serially diluted serum.

T F 2. The ox hemolysin test has been found to be quite specific and sensitive but time-consuming.

T F 3. In tests with papain-treated sheep erythrocytes, the proteolytic enzyme, papain, has been found to destroy the receptor sites for IM antibodies on sheep erythrocytes.

T F 4. In tests with papain-treated sheep erythrocytes, both the papainized cells and untreated cells agglutinate in infectious mononucleosis.

T F 5. Horse erythrocytes have been found to be more sensitive with fewer false positives than sheep erythrocytes for detection of IM antibodies.

T F 6. Both formalized cells and cells preserved in 3.8 percent citrate give unacceptable results.

T F 7. An important consideration in using any of these slide tests is to follow the manufacturer's instructions at all times.

T F 8. Febrile agglutination tests are considered to be better than titrations for following the course of IM.

2-2. Febrile Agglutination Tests

Prior to the development of modern bacteriological techniques, the cultivation of many of the common bacteria was difficult and haphazard. Such an organism as *Salmonella typhi,* which is routinely cultured now, was rarely isolated in active infections. Carriers of *S. typhi* organism were even more difficult to identify because the organism was at one time the cause of great epidemics. Large segments of entire communities were at times affected by the disease.

Typhoid fever produces characteristically a very high fever. The disease is classified, along with several other fever-producing illnesses such as brucellosis, tularemia, and the various rickettsial infections, into a category known as the *febrile diseases.* Since culture techniques for identifying fever-producing microorganisms were not very practical, alternate methods of testing for presence of infection were developed by Weil, Felix, Widal, and others. These methods tested for the presence of antibodies against the various microorganisms causing a particular infection. These serological methods are referred to as *febrile agglutination* tests. In this section, we will discuss these tests with particular emphasis of the "classical tests," the antigens and antibodies involved, and specificity of the reaction.

426. Specify the types of specimens required for bacterial agglutination tests; cite organisms causing febrile illness; and point out the diseases for which bacterial agglutination tests have been used for diagnoses.

Bacterial Agglutinins. Tests for bacterial agglutinins should be done on acute and convalescent specimens. In some febrile illnesses the antibody responses does not appear until after the first week. Ten days to two weeks or more may be required before a significant response can be detected. Tests on samples collected at staggered intervals provide more meaningful test results. In some instances patients who have had febrile diseases such as typhus or brucellosis show an anamnestic reaction (incidental stimulation of a prior immunization) when tested for typhoid. In an anamnestic reaction the presence of typhoid organisms restimulates the body to produce antibodies against previous typhus or brucellosis infections. The presence of these antibodies tends to confuse the interpretation of the test unless you consider the patient's history and know some of the characteristics of the causative agents.

Salmonella organisms cause a group of febrile illnesses important because of their high morbidity and mortality. These diseases are very prevalent where sanitary standards are low. The diseases occur frequently in Central Europe, the Middle East, and Asia. They still occur occasionally in rural areas of the United States and Canada. Typhoid fever is caused by *Salmonella typhi.* Paratyphoid fever is caused by several strains including *S. paratyphi A, S. paratyphi B,* and *S. choleraesuis.*

Bacterial agglutination tests have been used in the diagnosis of such diverse diseases as plague, brucellosis, tularemia, typhus, Rocky Mountain spotted fever, typhoid fever, and others. For some of these diseases, newer tests (for example,fluorescent antibody tests) have nearly replaced febrile agglutination tests. In addition, many of these diseases are rare in the United States today. However, because of the possibility of encountering these diseases with increased frequency in other parts of the world, the more common bacterial agglutination tests are included in our discussion.

Exercises (426):

1. What two types of samples should be collected for bacterial agglutinin tests in order to provide more meaningful test results?

2. What is an anamnestic reaction in terms of the body response to febrile diseases?

3. When the presence of typhoid organisms stimulates antibodies against previous typhus or brucellosis infections, these antibodies tend to confuse the test. What two key factors should the technician be aware of?

4. What organisms cause paratyphoid fever?

5. What are some diseases for which bacterial agglutination tests have been used?

427. Define the Widal reaction and cite its importance; specify the two groups of Salmonella antigens and the characteristic differences.

Widal Reaction. The first serologic test used to diagnose human disease was for the detection of agglutinins in the serum of typhoid fever cases. These sera agglutinated suspensions of the typhoid bacillus, *Salmonella typhi.* This reaction is the so-called Widal reaction—named for one of the early discoverers of this phenomenon. As currently used, the Widal reaction is a general term for agglutination tests for all salmonelloses, including typhoid and paratyphoid fevers.

The Widal procedure shows a very important fact about serology. This fact is that antigen-antibody reactions take place only in electrolytic solutions. The saline used to reconstitute the specimen is vital to the reaction. The number of bacteria in the antigen suspension also plays a significant part in the reaction. In most commercially prepared bacterial antigens, the bacteria are killed, usually with heat or phenol. The number of bacteria required to give a good reaction at a 1:80 dilution (the approximate dilution achieved in the original Widal test) varies with the strain of organism. Sometimes the number of organisms in a suspension is adjusted photometrically. This allows very precise antigen preparation for optimum antigen-antibody reaction.

Bacterial agglutination tests for the fevers caused by *Salmonella* organisms usually determine the presence or absence of antibodies against the following antigens: (1) *S. typhi O* and *H*, (2) *S. paratyphi A*, and (3) *S. paratyphi B*. The incidence of infections due to *Salmonella typhi* has fallen off markedly in the United States, while the incidence of infection due to other Salmonella species has increased. Other *Salmonella* organisms can be tested for if desired. The decision to use other antigens depends on the prevalence of a particular group or strain within the testing area.

The O antigen is a somatic (cell body) antigen. It is protein in nature, is heat-resistant, and can be heated at boiling temperatures for more than 1 hour without damage. Agglutination produced when O antigen and antibody react is hard, granular, and sometimes difficult to break up even with vigorous shaking. The H antigen is a flagellar antigen derived from components in or on the flagella of the organisms. Flagellar agglutination produces a fluffy, cottonlike aggregate that is easily dispersed on shaking. The reaction results in loose intertwining of the flagella into soft knots in the presence of anti-H antibody.

The reason we test for two different antibodies (anti-O and anti-H) in typhoid fever is that the strain of *Salmonella* in the test produces O antigens that are very highly group specific, and the antibodies produced as a result of *Salmonella typhi* infection will reach with all Group D *Salmonella* and occasionally with a few members of other groups.

The O antigens are especially important because the titer of O agglutinins rises earlier in the disease and then drops faster than H agglutinins. Testing for H agglutinins is usually unimportant because titers are slow to rise in a disease and then add to the confusion by remaining elevated for several years. Immunization also results in elevated H agglutinins while O agglutinins are not affected as much. Unfortunately, however, many manufacturers still customarily include H antigens in their febrile antigen kits so some discretion must be used by the serologist in selecting the antigens for his test battery.

Currently only about 20,000 cases of salmonellosis are reported in the United States each year. Most of these cases are due to salmonellae of Groups B, C_1, C_2, and D with Group B being the most frequent. Only about 400 cases of the most severe salmonellosis, typhoid fever, occur each year in the United States. Other groups of salmonellae are more prevalent in other countries. Therefore, the serologist must also consider the prevalent strains for each given locality in selecting his test antigens.

Exercises (427):

1. What is the Widal reaction?

2. What specific type of solution in the Widal tests enables the antigen-antibody reactions to take place?

3. In most commercial kits how are the bacteria killed?

4. The number of bacteria required to give a good reaction at a 1:80 dilution varies with what factor?

5. The incidence of infection has increased due to what Salmonella species in the United States?

6. Which of the Salmonella antigens is described as a somatic antigen, protein in nature, and heat resistant?

7. Which of the salmonella antigens produces an antigen-antibody reaction that is hard, granular, and sometimes difficult to break up even with vigorous shaking?

8. Why should care be exercised when reading flagellar antigen-antibody reactions?

9. Why are O antigens especially important?

10. Most cases of salmonellosis in the United States are due to what groups of salmonellae?

11. What group of salmonellae is the most frequent cause of salmonellosis?

428. Define the Weil-Felix reaction; idenify the type of organism producing antibodies in the test and the three strains of Proteus commonly used; and list two other bacterial antigens which may be included in the febrile agglutinin batteries.

Tests for Rickettsial Agglutinins. Some of the rickettsial diseases stimulate the production of bacterial agglutinins against certain nonmotile *Proteus* OX strains. This is referred to as the Weil-Felix reaction. This practical application of cross-reactivity has proven useful because rickettsiae require special techniques, such as animal inoculations, to isolate the organisms. Although fluorescent antibody and other serologic techniques can be used for the serodiagnosis of certain rickettsial diseases, the average clinical laboratory still makes use of *Proteus* agglutination tests for these diseases. Three strains of *Proteus* are commonly used as antigens. They have been designated *Proteus*, OX-19, OX-2, and OX-K. The production of agglutinins against these three strains varies from disease to disease and even from patient to patient. However, at times the agglutinins may be detected in the serum just a few days after the onset of disease, so these tests have proven very useful.

In the strain of *Proteus* bacillus designated OX-19, the "O" means the antigen is a somatic antigen of this nonmotile strain. The "X-19" refers to the particular strain number.

Two other strains of the *Proteus* bacillus give positive agglutination tests with other rickettsial organisms. In some cases, sera from several different kinds of rickettsial infections will cause the agglutination of the same strain of *Proteus*. The other two types of *Proteus* that react with sera from rickettsial infections are labeled OX-2 and OX-K. Table 2-4 shows the reactions of *Proteus* antigens in rickettsial infections.

Some serums will contain predeveloped, low-titer antibodies against one or several of the *Proteus* strains. The titer of these antibodies should not increase significantly in non-cross-reacting rickettsial infections. You can eliminate these preformed antibodies as a source of error by taking specimens for testing several times over a period of several days. A significant rise in titer in this test is more diagnostic than a single specimen with high titer. Be extremely careful in handling specimens from suspected rickettsial infections, especially in a laboratory that attempts to culture the organisms. You can contract a fever quite easily through mishandling of laboratory specimens and refuse.

TABLE 2-4
RICKETTSIAL DISEASES

ORGANISM	DISEASE	CARRIER	REACTION		
			OX19	OX2	OX-K
R. prowazekii	TYPHUS Epidemic Brill's Disease	BODY LOUSE	 + -	 -/+ -/+	 - -
R. mooseri	ENDEMIC TYPHUS	FLEA	+	-/+	-
R. tsutsugamushi	SCRUB TYPHUS	MITES	-	-	+
	SPOTTED FEVER GROUP	TICK			
R. rickettsii	ROCKY MOUNTAIN SPOTTED FEVER		Variable		-
R. conorii	BUOTONNEUSE FEVER		"		"
R. australis	QUEENLANDS TICK-TYPHUS		"		"
R. sibiricus	NORTH ASIAN RICKETTSIOSIS		"		"
R. akari	RICKETTSIALPOX	MITE	-	-	-
C. burnetii	Q FEVER	TICKS (Airborne fecal material)	-	-	-
R. quintana	TRENCH FEVER	BODY LOUSE	-		-

Brucellosis-Tularemia Agglutinins. Two other bacterial antigens that may be included in febrile agglutinin batteries are somatic (O) antigens of *Brucella abortus* and *Francisella (Pasteurella) tularensis*. Both brucellosis and tularemia are relatively rare diseases in the U.S. with only about 200 cases of each reported each year. However, these diseases must be considered when undiagnosed febrile diseases occur.

Both of these diseases are difficult to diagnose by bacteriological methods. Even if an organism is isolated, definitive identification is still a time-consuming project. Part of this problem arises from the relative rarity with which the bacteriologist encounters these organisms. Therefore, most cases of brucellosis and tularemia are detected serologically.

The strain of *Brucella abortus* used as the febrile antigen will detect agglutinins produced against all three species infecting man, namely, *B. suis* and *B. melitensis* as well as *B. abortus*. In addition to this across-reactivity, significant cross-reactivity is seen between *Brucella* and *Francisella* and vice versa. Consequently, titers using both antigens will have to be performed in order to obtain more definitive information on the actual disease agent causing the antibody production.

The actual selection of the antigens to be used in the febrile agglutinin battery must be made by the serologist. This selection must take into consideration the disease prevalence of the geographic locality of the patient's history and symptoms as well as the availability of the antigens. Table 2-5 lists some suggested antigens for use in the U.S. If the patient has recently traveled to the Far East, *Salmonella* O Group A and *Proteus* OX-K should be considered as possible additions. Travel to other parts of the world may suggest additional modifications of the battery.

TABLE 2-5
SUGGESTED FEBRILE ANTIGENS FOR USE IN THE CONTINENTAL U.S. (NEW)

Suggested Antigen	Most Frequent Causative Organisms
Salmonella Group D somatic	*Salmonella typhi* *S. enteritidis*
Salmonella typhi flagellar d	*S. typhi*
Salmonella Group B somatic	*S. typhi-murium* *S. heidelberg*
Salmonella Group C somatic	*S. infantis* *S. newport*
Proteus OX-19, OX-2	*Rickettsia typhi* *R. rickettsii*
Brucella abortus	*Brucella abortus* *Brucella suis* *Francisella tularensis*

Exercises (428):

1. What is the Weil-Felix reaction?

2. Does the Weil-Felix test detect anti-*Proteus* antibodies?

3. What are the three strains of *Proteus* commonly used in the test?

4. In the strain of *Proteus* bacillus designated OX-2, what does the "O" mean?

5. What type of results in the titer is considered more diagnostic?

6. What other two bacterial somatic antigens may be included in the febrile agglutinin batteries?

7. How are most cases of brucelloses and tularemia detected?

8. What factors must be considered in the selection of antigens to be used in the febrile agglutinin battery?

9. What two possible antigens should be considered if the patient has recently traveled to the Far East?

429. Indicate whether given statements correctly reflect techniques and guidelines for the tube and slide methods for agglutination tests.

Tube Versus Slide Tests. As mentioned before, you can perform any of the febrile agglutination tests more accurately in a test tube than on a slide. The difficulty in doing tube tests is the large amount of time involved in diluting and pipetting the specimen and the long incubation time. You can cut diluting and pipetting to a minimum by preparing the orignal dilutions in large lots and distributing this to other tubes set up for specific tests. For example, in testing for *Salmonella* you can eliminate having to make one dilution for the O antigen test and another for the H antigen test. You can also save time by using an automatic pipetting apparatus to dispense the antigen suspension.

The original slide procedures utilized a suspension of the organism as antigen and were not very reactive nor accurate. Many antigen suspensions today consist of antigen adsorbed onto latex principles. These suspensions are very reactive and yield tests quite comparable to tube tests. They are particularly recommended as screening procedures. Several manufacturers recommend their antigens for slide quantitative tests in which the specimen is serially diluted and the tests are performed at approximately the same dilutions as in tube tests. Although the quantities of serum placed on the slide have been correlated with the serum dilutions used in the tube test, for most accurate results, the tube test should be performed on all positive—and especially weakly positive—slide tests. Only completely negative (that is, less than 1:20) results should be reported solely on the basis of the slide test.

Exercises (429):

Indicate whether each of the following statements is true (T) or false (F). If you indicate false, correct the statement.

T F 1. Some difficulties encountered in doing tube tests are the large amount of antigen required and the unreliably short incubation time.

T F 2. Diluting and pipetting can be cut to a minimum by preparing the original dilution in large lots and distributing this to other tubes set up for specific tests.

T F 3. Antigens consisting of adsorbed latex particles are very reactive and yield tests quite comparable to tube tests.

T F 4. Several manufacturers recommend their adsorbed latex antigen suspensions for slide quantitative tests in which serial dilution and performance approximates those of the tube tests.

T F 5. On all positive and weakly positive slide tests, a tube test need not be performed for most accurate results.

T F 6. Only completely negative results should be reported on the basis of the slide test.

2-3. Other Agglutination Tests

Cold agglutination tests and *Streptococcus* MG tests are other agglutination tests performed in most serology laboratories. These tests are diagnostic for specific antibodies found consistently in primary atypical pneumonia (PAP). The cold antibody test is a hemagglutination test, and the *Streptococcus* MG test is a bacterial agglutination test. In both cases we identify antibodies in the patient's serum. In this section we shall discuss these tests and those conditions in which they are significant.

430. Indicate whether given statements correctly reflect the cause of primary atypical pneumonia (PAP) and conditions in which cold agglutinins are present.

Primary Atypical Pneumonia. The cold agglutinin (or cold antibody) is present in a great many illnesses. It has been detected in primary atypical pneumonia (PAP), pregnancy, *Staphylococcus* bacteremia, tonsillitis, cirrhosis of the liver, influenza, and many other conditions.

Primary atypical pneumonia (PAP) is a term used to describe a syndrome that is different from the "typical" labor pneumonia caused by *Streptococcus (Diplococcus) pneumoniae*. For many years, PAP was a synonym for viral pneumonia, because the syndrome was to be caused by various viruses. In fact, some are due to viruses; however, since the characterization of the Eaton agent (long thought to be a virus) as a pleuropneumonia-like organism, *Mycoplasma pneumonia*, PAP has been used primarily for Eaton agent pneumonia. More specifically, the disease is called cold agglutinin positive PAP, as well as Eaton agent pneumonia, to differentiate it from other atypical pneumonias. This discussion is limited to the serodiagnosis of cold agglutinin-positive primary atypical (Eaton agent) pneumonia. *Mycoplasma pneumonia* may be cultured, and the patient may show a rise in antibody titer even in the absence of symptoms. The isolation and identification of *Mycoplasma* is a rather slow process; therefore, most cases of primary atypical pneumonia (Eaton agent) will be diagnosed serologically.

Exercises (430):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. Cold agglutinins may be detected in primary atypical pneumonia, pregnancy, staphylococcus bacteremia, and influenza.

T F 2. Primary atypical pneumonia is a term used to describe certain cases of lobar pneumonia.

T F 3. Primary atypical pneumonia is strongly associated with the pleuropneumonia-like organism, *Streptococcus pneumoniae*.

T F 4. More specifically, the disease is called cold agglutinin positive PAP as well as Eaton agent pneumonia.

T F 5. The isolation and identification of *Mycoplasma* is a rather rapid process and serodiagnosis is sometimes unnecessary.

431. Identify the serologic tests in terms of the specific antibodies detected in the diagnosis of primary atypical pneumonia.

Tests for Primary Atypical Pneumonia. There are several types of antibodies produced during the disease. Consequently, several serologic tests have been developed to detect these antibodies. Some of the tests are beyond the capability of the average clinical laboratory; but three of these newer tests will be described briefly because they may become more prevalent in the future. As with most serologic tests, detecting a rise (fourfold) in antibody titer is most significant.

Immunofluorescent antibodies. These antibodies are detected using the indirect fluorescent antibody technique with sections of lungs from chick embryos infected with *Mycoplasma pneumoniae* serving as the antigen. This test has proven to be highly specific and sensitive for diagnosing this disease, but the limitations for the average clinical laboratory are obvious. To date, the use of *M. pneumoniae* grown in culture as the antigen for this test has been less successful than using infected chick embryos.

Growth-inhibiting antibodies. These antibodies may be detected by their ability to inhibit the growth of *M. pneumoniae* in culture. The organism is added to the patient's serially diluted serum in a medium containing tetrazolium. If these antibodies are present, the organism does not grow, and the tetrazolium is not reduced to a red form. The main limitations of this procedure are the need for live *M. pneumoniae* and the organism's slow growth—the test takes about 1 week to complete. Despite these limitations, this test has proven to be highly sensitive and specific for the seriodiagnosis of PAP.

Indirect hemagglutination antibodies (IHA). These antibodies are detected by mixing the patient's serially diluted serum with tanned sheep RBCs that have been coated with sonicated *M. pneumoniae*. Agglutination of the RBCs indicates the presence of antibodies. This test is usually considered to be too sensitive because the antibody titer rises to high levels so early in the disease that a subsequent rise in titer may be undetectable. This test has been adapted to the Microtiter system and may soon acquire a prominent role in the serodiagnosis of PAP; however, the procedure will not be included in this text.

Streptococcus MG agglutinins. About half or less of the patients with PAP produce bacterial agglutination antibodies (agglutinins) against *Streptococcus* MG. Generally the presence of these agglutinins parallels the severity of the disease, but their absence does not rule out the disease due to frequent false negatives. On the other hand, only rarely are false positives encountered against the *Streptococcus* MG antigen, but the true relationship of *Streptococcus* MG with *M. pneumoniae* is unknown. Both organisms may be simultaneously isolated from cases of PAP, but there appears to be no direct relationship of *Streptococcus* MG with the disease. Even though the significance of these *Streptococcus* MG agglutinins is unknown and they are not always produced, their detection still provides a simple aid in the diagnosis of PAP.

Cold hemagglutinins. These are antibodies that agglutinate RBCs (including the patient's RBCs) in the cold, but the RBCs disperse when the mixture is heated to 37° C. The usual test temperature is about 4° C, but the temperature at which the antibodies react varies with each patient. Some patients may produce antibodies that react at temperatures as high as 35° C. This variability of reaction temperatures is especially important in specimen collection, because the patient's RBCs may absorb the antibodies from the serum before the serum is separated from the cells if the blood has cooled too much. Proper blood collection for this test is important.

Unfortunately, not all patients produce detectable cold agglutinins during the course of Eaton agent pneumonia. Although cold agglutinin production seems to parallel the severity of the disease, various studies have shown that as few as 30 percent to as many as over 90 percent of patients actually produce these antibodies. In addition, several other disease conditions result in the production of cold agglutinins, but only rarely are these antibodies produced in a pneumonia other than PAP.

Even with the false negatives and false positives the test for cold agglutinin still provides a simple laboratory test for PAP. The test is more valuable when performed in conjunction with another test for PAP. The most simple combination is to perform this test along with the *Streptococcus* MG agglutination test.

Exercises (431):

Match each type of antibody detected in seriologic tests for primary atypical pneumonia in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. These antibodies are detected using the indirect fluorescent antibody technique with sections of lungs from chick embryos infected with *mycoplasma pneumoniae* serving as the antigen.
___ 2. In the tests detecting this antibody, the organism is added to the patient's serially diluted serum in a medium containing tetrozolium.
___ 3. The main limitations of this procedure are the need for live *M. pneumoniae,* the organism's slow growth, and the testing takes about 1 week to complete.
___ 4. These antibodies are detected by mixing the patient's serially diluted serum with tanned sheep RBCs that have been coated with sonicated *M. pneumoniae.*
___ 5. The test is usually considered too sensitive because the antibody titer rises to high levels so early in the disease that a subsequent rise may be undetectable.
___ 6. Generally, the presence of these agglutinins parallels the severity of the disease.
___ 7. Some patients may produce these antibodies that react as high as 35° C.
___ 8. Rarely are false positives encountered against the antigen used in detecting these antibodies.
___ 9. Not all patients produce these detectable antibodies during the course of Eaton agent pneumonia.
___ 10. The most simple combination is to perform this test along with the *Streptococcus* MG agglutination test.

Column B

a. Cold hemagglutinins.
b. Growth-inhibiting antibodies.
c. Immunofluorescent antibodies.
d. Streptococcus MG agglutinins.
e. Indirect hemagglutination antibodies (IHA).

432. Indicate whether given statements correctly reflect the technique for blood collection, processing, and general procedures for cold hemagglutinin titration.

Collecting Blood for Cold Hemagglutinin Titration. Although no special precautions are needed for collecting serum for most serologic tests, collecting serum for cold agglutinins is an exception. This is because the cold agglutinins can be absorbed from the serum by the patient's RBCs as the blood specimen cools. To prevent this, the blood must be maintained at 37° C until the serum can be separated. Prewarm the syringe and/or vacuum tube (without anticoagulant) to body temperature by holding them tightly in the hand for 2 to 3 minutes. Also, prewarm the tube that will receive the blood if a syringe is to be used. Keep the blood at body temperature (for example, held tightly in the hand) and put the tube in a 37° C water bath or incubator. Allow the blood to clot at 37° C for about 30 minutes, ring the clot, and centrifuge the contents IMMEDIATELY for about 3 to 5 minutes. IMMEDIATELY separate the serum, making sure that it is cell-free. The serum should be tested as soon as possible after collection; however, cell-free serum may be refrigerated or frozen until it is tested.

Procedure for Cold Hemagglutinins. Human group O cells are the source of antigen. Wash the cells and resuspend them as a 1-percent suspension. Serially dilute the patient's serum with saline. The dilution progresses 1:4, 1:16, etc. After setting up the test, place it in a refrigerator at 2° to 4° C overnight. A preliminary reading may be made after 1 hour in the refrigerator. High titered sera will usually show significant reactions in 1 hour. Immediate reading of test results after removing the tubes from the refrigerator is necessary, since warming may cause agglutination to disappear. Titers of 1:16 or less are considered nonsignificant. Titers of 1:32 or 1:64, if accompanied by a typical clinical picture, are significant. Higher titers on a single convalescent specimen are, of course, more diagnostic. The test is much more relevant if both acute and convalescent specimens are tested. A fourfold increase in titer is indicative of primary atypical pneumonia. Titers tend to vary with the severity of the disease; the more severe the disease, the higher the titer. However, for some unexplained reason some cases of severe disease show no elevation of cold antibody titer during the course of the illness.

Exercises (432):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. Since cold agglutinins can be absorbed from the serum by the patient's RBCs, the blood must be maintained at 2° to 4° C until the serum can be separated.

T F 2. The tube of blood may be kept at body temperature by holding tightly in the hand before placing in a 37° C water bath or incubator.

T F 3. After the blood has been centrifuged any delay in the separation of the serum is permissible.

T F 4. Human group AB cells are the source of antigen in the test.

T F 5. The test is placed in the refrigerator overnight, but preliminary reading may be done in 1 hour.

T F 6. After overnight setting in the refrigerator, then removal, immediate reading of the test is not necessary since the agglutination is permanent.

T F 7. Titers of 1:32 or 1:64, if accompanied by a typical clinical picture, are significant.

T F 8. The test is more relevant if both acute and convalescent specimens are tested.

CHAPTER 3

Latex-Fixation, Precipitin, and ASO Tests

RECENT RESEARCH and development of new serological techniques have been motivated by two requirements. The first is for tests that will quickly and easily diagnose difficult or previously undetectable diseases. The second is for tests that can be performed rapidly with a minimum of supplies and equipment. Latex-fixation and related tests have generally met these requirements. Most of these tests are prepackaged in kit form. The tests can be performed rapidly, and in some instances accuracy is greatly improved. Newer tests for rheumatoid arthritis are good examples of this. A serum specimen can be screened in 2 or 3 minutes. The older procedures required several hours to set up and were somewhat lacking in specificity.

Although the newer slide tests are easy to perform and itnerpret, the principles that govern their reaction are just as involved as the older procedures. In this chapter we shall discuss the components and theory of the reaction of latex-fixation and precipitin tests. Also, we will review the antistreptolysin test with mention of a newer slide method for detection of antibodies to streptococcal exoenzyme, Streptozyme.

3-1. Latex-Fixation Tests

To most laboratory technicians, when we say "latex-fixation" it is understood that we are talking about a test for rheumatoid arthritis. Latex particles may, however, be used in a variety of applications. They are used in the diagnosis of trichinosis and in the rapid slide test febrile agglutination procedures. While such test kits are supplied with charts for interpreting test results, it is desirable for you to know why the reaction occurs. With this information, quality control of the procedure is easier and the limitations of the test are easier to understand.

433. Indicate whether given statements correctly define rheumatoid arthritis and the significance and characteristics of rheumatoid factors.

Rheumatoid Arthritis. Rheumatoid arthritis is a chronic inflammatory disease of unknown etiology affecting the joints and synovial membranes. Early symptoms of joint pain and muscular stiffness may occur at any age, with females more frequently affected than males. These early symptoms are not unique to rheumatoid arthritis, so several other diseases must be ruled out in the differential diagnosis. Later in the disease, subcutaneous nodules are frequently noted over bony prominences. The disease may ultimately progress to severe skeletal deformity and complete immobilization of the affected joints.

Rheumatoid Factors. Depending on the test method used, about 80 percent of classical cases of rheumatoid arthritis have anti-gamma-globulin antibodies in their serum. These antibodies are mainly against IgG and react with the gamma globulin from several species of animals, as well as from the patient. Because these antibodies are most frequently associated with rheumatoid arthritis, they are called rheumatoid factors or, simply, RF.

Rheumatoid factors were originally thought to be a single macroglobulin related to IgM immunoglobulin, but they are now known to be, in fact, IgM immunoglobulin along with small quantities of IgG and IgA. The presence of rheumatoid factors in IgM, IgG, and IgA is further evidence that these factors are antibodies. In effect, they are antibodies against antibodies.

Unfortunately, the mere presence of rheumatoid factors in the serum does not confirm a diagnosis of rheumatoid arthritis, because a varying percentage of several other diseases may produce detectable levels of so-called rheumatoid factors. In addition, a negative test for rheumatoid factors does not rule out the possibility of rheumatoid arthritis, because about 20 percent of actual cases have negative tests. The presence or absence of rheumatoid factors also varies with the age of the patient, with children less apt to produce the factors, while older individuals are more frequently postivie—even in the absence of rheumatoid arthritis. Despite these variations in production, serotests for the detection of rheumatoid factors in the serum are useful in the diagnosis of rheumatoid arthritis. Detecting increases in the titer of these factors is especially significant.

Exercises (433):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. Rheumatoid arthritis is an acute inflammatory disease involving tissue lesions such as skin, mucosa, kidney, or brain.

T F 2. Early symptoms of joint pain and muscular stiffness are unique to rheumatoid arthritis.

T F 3. About 80 percent of classical cases of rheumatoid arthritis have anti-gamma-globulin antibodies in their serum.

T F 4. These antibodies, called rheumatoid factors, are mainly against IgM and react with gamma globulin from several species of animals, as well as from the patient.

T F 5. The presence of rheumatoid factors in IgM, IgG, and IgA is further evidence that these factors are antibodies.

T F 6. A negative test for rheumatoid factors rules out the possibility of rheumatoid arthritis.

T F 7. The presence or absence of rheumatoid factors also varies with the age of the patient.

T F 8. Since the disease has been already diagnosed, detecting increases in the titer of these factors is of little significance.

434. Identify the tests for rheumatoid arthritis in terms of the given principles, reagents, and sources of error.

Tests for Rheumatoid Factors. Several types of tests have been developed for the detection of rheumatoid factors in the serum. Some of these tests, such as those involving radioimmunoassay, are very complex and, therefore, will be impractical for the average clinical laboratory. Most of the practical tests are based on the passive agglutination of either erythrocytes or inert particles coated with gamma globulin from animals or humans. The erythrocytes most frequently used are either fresh or preserved sheep cells, while polystyrene latex particles are the most commonly used inert particles. In general, the latex tests are more sensitive (more false positives with other diseases) than the erythrocyte tests, but a positive erythrocyte test is more likely to indicate rheumatoid arthritis. Examples of both types of tests are included in this discussion. Most of these tests are available commercially in kit form.

Latex Macroscopic Tube Test. In the latex macroscopic tube test, a suspension of globulin-coated latex particles is added to serially diluted serum in test tubes. After incubation at times and temperatures specified by the kit manufacturers, the reciprocal of the highest dilution of serum to cause agglutination of the latex particles is reported s the titer of rheumatoid factors in the serum.

Latex-fixation tests performed in test tubes are more accurate than slide test screening procedures. It is important that you run appropriate controls with each batch of tests, especially since you will be using a stored preserved antigen which can become slightly lumpy after a period of storage. While slide screening procedures are adequate in most instances, there will be occasions, such as in observing the effect of therapy, when precise titrations are required. The tube test will then be the method of choice.

Sensitized Sheep Cell Slide Test. In this procedure, the patient's serum is mixed with stabilized sheep erythrocytes that have been coated (sensitized) with gamma globulin. Agglutination of the erythrocytes indicates the presence of rheumatoid factors in the patient's serum.

The Rose test or some modification of the Rose test was for many years the test of choice. This test employed sheep erythrocytes sensitized by exposing them to small amounts of rabbit anti-sheet globulin. A normal control of unsensitized cells was run at the same time. When the sensitized and unsensitized cells were exposed to dilutions of serum from an arthritic patient, agglutination of a higher titer would occur in the sensitized cells than in the unsensitized cells. A significant number of arthritic patients did not give this pattern or gave variable test results.

Modifications were made to overcome the deficiencies of the Rose test. Human erythrocytes were substituted with no appreciable improvement of the test. Tannic acid was tried as a sensitizing agent. Collodion particles and other inert carriers were tried. The sensitized sheep cell slide test represents a modification of the Rose test.

Some sources of error must be considered when using this test. They are listed accordingly:

(1) Do not freeze the erythrocyte reagent because hemolysis may occur.

(2) Be sure to bring all reagents and sera to room temperature before testing.

(3) The possibility of the presence of heterophile antibodies must be considered in all positive tests.

(4) Undiluted, unheated serum at room temperature must be used for this test.

Latex Slide Screening Test. In this test a 1:20 dilution of patient's serum in glycine-saline buffer diluent is mixed on a slide with a suspension of globulin-coated polystyrene latex particles.

Agglutination (macroscopic clumping) of the latex particles indicates the presence of rheumatoid factors in the patient's serum.

Polystyrene latex particles are used in the several tests for rheumatoid arthritis currently in use. These particles are soaked in normal gamma globulin from humans or any of seven other animals. The latex particles absorb some of the gamma globulin and the excess is washed off. The coated particles are reconstituted to a specific turbidity in buffered saline. Spectrophotometers or related instruments are used to adjust the turbidity of the latex suspension so that an optimum concentration can be obtained.

The test is performed by mixing a quantity of the latex-antigen suspension with serum. If anti-gamma-globulin antibodies are present in the test specimen, the latex particles are agglutinated. While the observable reaction appears to be agglutination, the reaction that actually takes place is a precipitin reaction. The latex particles absorb a soluble antigen. Reactions between soluble antigens and antibodies result in precipitation or the formation of soluble antigen-antibody complexes. The gamma globulin (antigen) used in this test is soluble. We speak of the test as a *latex-fixation* because the antigen (γ-globulin) is fixed (absorbed) by the latex carrier particle.

Eosin-Latex Slide Screening Test. A modification of the basic, simple latex-fixation test is the eosin latex-fixation test of Singer and Plotz. Patient's serum (or whole blood) is mixed with a drop of eosin solution on a slide. Then, a drop of a suspension of uncoated latex particles is mixed with the serum-eosin mixture. Spontaneous agglutination of the latex particles indicates the presence of rheumatoid factors in the serum. The addition of the eosin solution before the latex particles reduces the incidence of false positive tests.

Although this test was designed to detect rheumatoid arthritis, it can be used in the diagnosis of a variety of collagen and tissue destruction diseases. Among the diseases giving a positive test are liver diseases, lupus erythematosus, hypergammaglobulinemia, sarcoidosis, and nonrheumatoid arthritis. Therefore, you will occasionally get a request to perform this test on nonarthritic patients. Perform the test and report the results in the same manner that you would if the specimen were from a rheumatoid patient. The significance of a positive test in the diseases mentioned above is determined by the physician on the basis of the clinical aspects of the patient's condition.

Exercises (434):

Match each test for rheumatoid arthritis in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. A positive test of this type is more likely to indicate the presence of rheumatoid factors.
___ 2. Might be done on occasions such as observing the effect of therapy.
___ 3. A suspension of globulin-like coated particles is added to serially diluted serum.
___ 4. Test employed sheep cells sensitized by exposing them to small amounts of rabbit anti-sheep globulin.
___ 5. A 1:20 dilution of patient's serum in glycine-saline buffer diluent is mixed on a slide with a suspension of globulin-coated polystyrene latex particles.
___ 6. Serum or whole blood may be mixed with a drop of reagent in this test.
___ 7. Addition of the solution in the test before the latex particles reduces the incidence of false positive tests.
___ 8. You may occasionally get a request to perform this test on nonarthritic patients.
___ 9. The possibility of the presence of heterophile antibodies must be considered in all positive tests.

Column B

a. Latex macroscopic tube test.
b. Latex slide screening test.
c. Sensitized sheep cell slide test.
d. Rose test.
e. Eosin-latex slide screening test.

435. Identify each of the given serologic tests in terms of its purpose, principle in utilizing latex particles, and other significant features.

Other Tests Using Latex Particles. Latex particles have been used as antigen carriers in a variety of commercially developed diagnostic test procedures. Soluble antigens are applied directly to the latex particles for absorption. Particulate antigens, such as suspensions of bacteria or macerated worms, are reduced to fine suspensions. These suspensions are prepared using any of several techniques, such as grinding, ultrasonics, or lysing. Commercial manufacturers have found ultrasonics to be the most satisfactory way of producing a smooth, even suspension.

C-reactive protein. Latex particles have also been used in tests to detect C-reactive protein. This protein appears in the blood in acute pneumococcal pneumonia. It has also been isolated in many other diseases and infections including myocardial infarction, streptococcal and staphylococcal infections, Hodgkin's disease, cirrhosis of the liver, and certain cancers. C-reactive protein can be identified by its ability to precipitate the somatic C-polysaccharide of rough stains of pneumococcus. Electrophoretically, the protein has properties similar to β γ-globulin. Its sedimentation constant is higher than serum γ-globulin. It has not been classified as an antibody.

To detect C-reactive protein, you use antiserum obtained by injecting rabbits with purified C-reactive protein. You use latex particles coated with the antiserum and mix them with the patient's serum. Visible agglutination occurs rapidly if C-reactive protein is present. In most instances, you run this test as a simple slide-test screening procedure. Another basic method, a precipitin test in capillary, involves the precipitation of CRP by anti-CRP antiserum.

Febrile and bacterial aggulatination tests. Manfacturers prepare febrile and other bacterial agglutination test antigens by soaking latex particles in extracts of broth cultures in which the desired microorganisms were cultured. The excess antigen is washed off and the latex suspension is diluted to desired turbidity. A drop or two of the commercially prepared diluted latex suspension plus a drop or two of the patient's serum provide an excellent rapid screening test; considerably less time is required than in the older tube test.

Tests for Systematic Lupus Erythematosus (SLE). Lupus erythematosus, a disease of connective tissue, causes the body of those affected to produce antibodies to desoxyribonucleic acid (DNA). The classical test for SLE is the demonstration of LE cells. In addition, two types of serologic tests have proven practical in the diagnosis of SLE. These serotests are important because only about 75 percent of SLE patients ever produce the typical LE cells, and the identification of these LE cells requires highly trained personnel. One type of serotest is based on the agglutination of nucleoprotein-coated latex particles and another on the detection of antinuclear antibodies by the indirect fluorescent antibody technique. In addition, complement fixation and several other serotests have been used in this disease.

Latex test for SLE. The most simple (but the least sensitive) test for SLE is the latex agglutination test using latex particles coated with nucleoprotein. The latex particles are carriers for the DNA antigen. Although a positive latex test is usually indicative of SLE, unfortunately, the test is only positive in about one-third of the cases of SLE. Despite these limitations, the speed and simplicity of this test have made it useful.

Fluorescent antinuclear antibody (ANA) test. Nearly 100 percent of cases of SLE and lower percentages of several other diseases produce one or more of the antinuclear antibodies. These antibodies are detectable by reacting the patient's serum with nucleated cells and detecting the antigen-antibody reaction following the addition of fluorescein-labeled, antihuman globulin. Although equipment for fluorescent microscopy is required for this test, this is the best screening test available for SLE. The titer of antinuclear antibodies may also be determined by this method.

Trichinosis. This intramuscular parasitic infection is another disease that is diagnosed almost exclusively by serologic means. Consequently, a multitude of serologic tests have been developed for this disease. One of the more widely accepted tests has been the aggulutination of antigen-coated bentonite particles. More recently, a test similar to the RPR card tests for syphilis has been described. In this card test, the antigen is adsorbed on cholesterol-lecithin crystals in a charcoal suspension. Some of the other tests that have been used in this disease are: complement fixation, precipitin, indirect hemagglutination, fluorescent antibody, and a variety of agglutination tests using particles, such as latex, collodion, and others. When latex particles are used, the commercially prepared product is a macerated extract of the worm absorbed onto latex particles. Mix a drop or two of the latex-antigen suspension with a drop or two of the patient's serum on a slide to make a simple, efficient screening test.

Amebiasis. The diagnosis of extraintestinal amebiasis, especially amebic liver abscess, is a complicated problem. A wide variety of serologic tests have been applied to this problem with varying degrees of success. Some of these tests are indirect hemagglutination, capillary precipitin, immunodiffusion, fluorescent antibody, latex, complement fixation, and immunoelectrophoresis tests. In addition, an intradermal skin test has been used.

Cryptococcosis. In addition to the usual tests for cryptococcal antibodies in a patient's serum, a latex test, using antibody-coated latex particles, has been developed to detect cryptococcal antigens in the patient's serum or CSF. The tests for antibodies are based on fluorescent antibody, indirect hemagglutination and whole-yeast cell agglutination. By comparing changes in titers of both antigen and antibody tests, valuable information is gained.

Histoplasmosis. In addition to immunodiffusion and skin tests, some of the tests that have been used in diagnosing histoplasmosis are fluorescent antibody, latex agglutination, and complement fixation tests. Care must be taken in interpreting the results of these serotests performed on patients who have had skin tests, because skin tests may stimulate the production of detectable levels of antibodies.

Gonorrhea. A recently developed latex test appears to be especially useful for the detection of chronic cases, such as asymptomatic females—the most difficult cases to diagnose by cultural methods.

Leptospirosis. Most serologic tests for this disease are based on the microscopic or macroscopic observation of agglutination of either live or formalinized spirochetes. Others that have been reported are fluorescent antibody, complement fixation, and latex tests.

Tuberculosis. Although the immunologically based skin tests for tuberculosis are widely used, serologic tests seem to have limited application. Some of the tests that have been tried are: complement fixation, immunodiffusion, indirect hemagglutination, and agglutination tests using coated latex, bentonite, or kaolin particles.

Hashimoto's Thyroiditis and Related Disorders. A high percentage of individuals with Hashimoto's tyroiditis and primary myxedema, as well as lesser percentages of cases with certain other thyroid disorders, produce antibodies against antigens from their own thyroid glands. Antibodies may be produced against microsomal antigens or thyroglobulin from the gland. Most of the practical serologic tests are directed toward detection of thyroglobulin antibodies. Some of these tests are immunodiffusions, indirect hemagglutination, latex agglutination, and fluorescent antibody tests. As with any biological system, these tests may be positive in the absence of disease or negative when disease is evident, but high titers are usually significant.

Fibrinogen Concentration. Although more definitive tests for this clotting factor are available in the chemistry and hematology manuals, a latex agglutination test is also available for estimating fibrogen concentrations.

Exercises (435):

Match each serologic test in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. The test uses antiserum obtained by injecting rabbits with the purified product.

___ 2. The somatic C-polysaccharide of rough strains of pneumococcus are precipitated.

___ 3. Test antigens are prepared by soaking latex particles in extracts of broth cultures in which the desired microorganisms were cultured.

___ 4. Uses latex particles coated with nucleoprotein; the latex particles are carriers for DNA antigen.

___ 5. Even though indicative, is positive only in one-third of the cases.

Column A

___ 6. Patient's serum reacts with nucleated cells and detected antigen-antibody reaction is followed by the addition of fluorescein, antihuman globulin.

___ 7. The commercially prepared product is a macerated extract of the worm absorbed onto latex particles.

___ 8. Tests for parasitic infection which may use latex particles.

___ 9. Test for mycotic infections which may use latex particles.

___ 10. Tests for bacterial and spirochetal infection which may use latex particles.

___ 11. Most of the practical serologic tests are directed toward detection of these thyroglobulin antibodies.

___ 12. More definitive tests for this factor are available in chemistry and hematology; a latex agglutination test is available for estimating the concentration.

Column B

a. Hashimoto's thyroiditis and related disorders.
b. Fibrinogen concentration.
c. Amebiasis.
d. Trichinosis.
e. Systemic lupus erythematosus (latex test).
f. Fluorescent antinuclear antibody test.
g. histoplasmosis.
h. Gonorrhea.
i. Cryptococcosis.
j. Leptospirosis.
k. Tuberculosis.
l. C-reactive protein.
m. Febrile and bacterial agglutination tests.

3-2. Precipitin Tests

The precipitin or precipitation reaction was first observed in 1897 and is one of the basic methods used to observe antigen-antibody reactions. There are a great many applciations where precipitin reactions can be of use. These include the estimation of antigens or antibodies in solution, in tissues, and in individual cells. The techniques devised to make these reactions visible include such diverse methods as diffusion, use of gel media, fluorescence, X-ray opacity, radioactive isotope tagging, or coupling the antigen or antibody with an enzyme. Most often a finely flocculent precipitate indicates a positive reaction. However, in some precipitin reaction, the reactions are not directly observable and one of the methods mentioned must be used to show that a reaction has taken place.

The precipitin reaction is not widely used in most USAF serological laboratories. However, with more remarkable advances in medical technology, these techniques have recently become available to those laboratories which used highly refined methods for identifying small amounts of antigen-antibody products. Immunodiffusion tests are of increasing interest to the serologist and blood banking technologist and warrant brief discussion in this section.

436. Indicate whether given statements correctly reflect the principle and characteristics of precipitation reaction and the capillary-tube precipitin tests.

Precipitation Reactions. Precipitation is the second-stage reaction in which the antibody (precipitin) reacts with a soluble antigen. The visible precipitate results from the sequential combination of antibodies and antigens until antigen-antibody aggregates large enough to be seen are formed. This formation of visible aggregates will occur only when optimum proportions of both antigen and antibody are present. Most precipitation reactions take several hours for a visible precipitate to appear. The tests are usually performed by one of two basic methods—in capillary tubes or by immunodiffusion in agar.

Capillary-Tube Precipitin Tests. In this technique, the soluble antigen and the antibodies (antisera) are introduced successively by capillary attraction into the tube so that the layers contact each other. After incubation in an upright position, the precipitate usually forms at the antigen-antibody interface or throughout the mixture if the proper antigen and antibody are present. This test is relatively insensitive since it detects only large amounts of antibodies. Another more obvious limitation of this method is that both antigen and antibody solutions must be clear, since cloudiness may interfere with reading the final results. This technique is used for the detection of C-reactive protein in the serum. Precipitin tests also have applications in bacteriology in the classification of streptococci.

Exercises (436):

Indicate whether each of the given statements is true (T) or false (F), and correct those that are false.

T F 1. Precipitation is the third-stage reaction in which the antibody (precipitin) reacts with a soluble antigen.

T F 2. The formation of visible aggregates will occur only when optimum proportions of both antigen and antibody are present.

T F 3. Precipitation tests are usually performed by one of two basic methods—capillary tubes or by agglutination in agar.

T F 4. In the capillary-tube precipitin tests, the soluble antigen and antibodies are introduced successively by capillary attraction into the tube so that the layers contact each other.

T F 5. After incubation in an upright position, the precipitate usually forms at the bottom of the tube mixture if the proper antigen and antibody are present.

T F 6. The capillary-tube precipitin test is relatively sensitive since it detects small amounts of antibodies.

T F 7. In the capillary tube precipitin test, both antigen and antibody solutions must be clear, since cloudiness may interfere with reading the final results.

437. Point out the basic principle of immunodiffusion tests, cite the types of tests and techniques used in each, and state the antibodies commonly detected by these tests.

Immunodiffusion Tests. There are many tests being developed which depend on the diffusion of either the antigen or antibody (or both) through a buffered agar before they react. Some of these methods require sophisticated laboratory equipment, such as for the detection of radioisotopes, and are beyond the scope of this text, but the increased sensitivity and specificity of these methods may soon make them practical. However, there are a few immunodiffusion tests which relay on the formation of a visible precipitate. Some of these are rather sophisticated tests too, but their importance warrants at least brief mention. In general, these tests are based on the migration of the antigen and/or antibody through the agar until optimum proportions of each are reacted and a precipitate appears. As usual, the appearance of the precipitate takes several hours, but a modification by combining diffusion with electrophoresis has speeded the reaction and has great promise for the future in serologic diagnosis.

Double diffusion tests. In double diffusion tests, the antigen and antisera are placed in separate wells cut into the agar, and each diffuses toward the other. A precipitate line appears where optimum proportions of the reactants are present. See figure 3-1. Although the technique yields only qualitative results, it is simple and can be used with mixed antigen systems or in many combinations of known-unknown situations. The test is referred to as the Ouchterlony test. It has been applied to the detection of antibodies in coccidioidomycosis.

Single radial diffusion tests. In this method, one reactant, usually the antibody, is incorporated into the agar. The antigen is placed in a well and diffuses radially into the agar. A ring of precipitate forms whose diameter is proportional to the antigen concentration (see fig. 3-2). Standard antigens of known concentrations may be tested at the same time. The diameter of the reactions of the standards can be plotted against their concentration; and then the concentration of unknown samples can be read directly from the standardization curve. This method is presently used for estimating the concentration of immunoglobulins (IgG, IgM) and complement (C3).

Immunoelectroosmophoresis (IEOP). This technique is also called counterelectrophoresis, counter-current electrophoresis, counterimmunoelectrophoresis, and others. Basically the principle is a combination of simultaneous diffusion and electrophoresis followed by precipitation at the point of optimal proportions of reactants. In the test, antigen and antibody are placed in separate wells

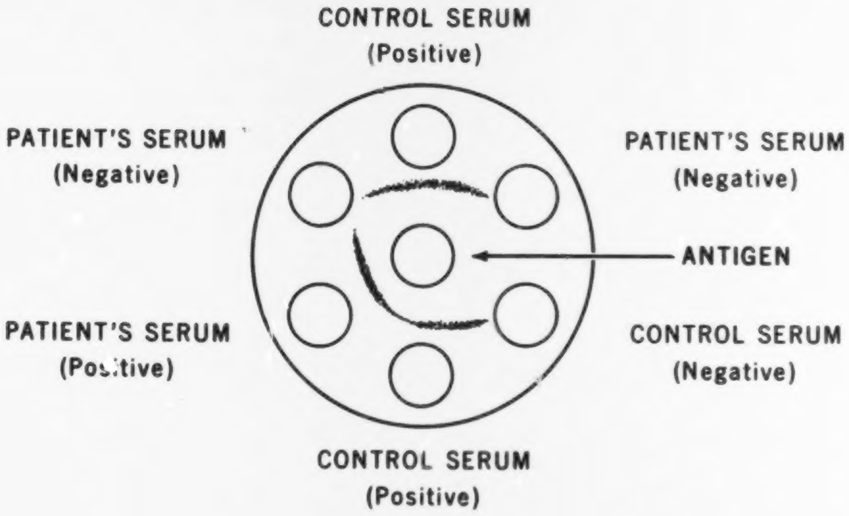
CONTROL SERUM
(Positive)
PATIENT'S SERUM
(Negative)
PATIENT'S SERUM
(Negative)
ANTIGEN
PATIENT'S SERUM
(Positive)
CONTROL SERUM
(Negative)
CONTROL SERUM
(Positive)

Figure 3-1. Double diffusion (Ouchterlony).

in the agar and an electrical current applied. The pH and ionic strength of the agar as well as the nature of the antigen and antibody determine the migration characteristics of the system. As applied to the detection of the hepatitis associated antigen (HB$_s$Ag) in the blood of potential blood donors, the antigen (HB$_s$Ag) is electrophoresed toward the known antibodies which are diffusing through the agar toward the antigen. A precipitate line appears when optimum proportions of each have reacted (see figure 3-3). The main advantages of IEOP over double diffusion tests are increased speed and the ability to detect very small quantities of antigen—both important considerations for the clinical serologist.

Exercises (437):

1. What is the basic principle of immunodiffusion tests?

2. Since the appearance of the precipitation takes several hours, with what technique is diffusion combined in order to speed up the reaction?

3. What type of diffusion is used in the Ouchterlony technique?

4. In the double diffusion tests, how are the reactants used?

5. The double diffusion test is applied to the detection of antibodies produced in what mycotic infection?

6. In the single radial diffusion test, where is the reactant used?

7. Where is the antigen placed and what type of reaction follows in the single radial diffusion?

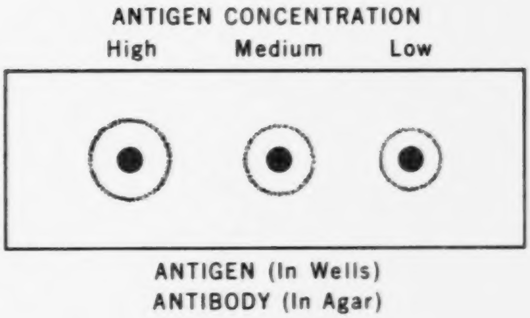
ANTIGEN CONCENTRATION
High
Medium
Low
ANTIGEN (In Wells)
ANTIBODY (In Agar)

Figure 3-2. Single radial diffusion.

8. What type of indicator is formed by the final reaction?

9. The single radial diffusion method is presently used for estimating what two complex-protein substances in serum?

10. What are two other terms that refer to immunoelectroosmophoresis (IEOP)?

11. What is the basic principle of IEOP?

12. Basically describe the test.

13. What factors determine the migration characteristics of the system?

14. What are the main advantages of IEOP over double diffusion tests?

15. To what important test for potential blood donors is the method of IEOP applied?

3-3. Antistreptolysin O

When certain strains of *Streptococcus* organisms infect the body they produce a substance which reacts enzymatically with red and white blood cells, thus destroying them. Group A strains of *Streptococcus* are the most frequent producers of this substance. It can also be produced by certain Group C and G organisms. This hemolytic substance (streptolysin) is really composed of two hemolysins. The first, streptolysin S, is an oxygen-stabile, nonantigenic lipoprotein. Due to its lack of antigenic activity, there is no practical way to measure it with available laboratory tests. The second hemolysin, streptolysin O, is oxygen-labile and a very good antigen. Procedures are available for measuring this hemolysin, and the test can be performed in laboratories of any size.

When the body is infected with one of the organisms which produce streptolysin, an immune response is initiated by the presence of streptolysin O. The body responds by producing antistreptolysin O antibodies. These antistreptolysin O antibodies are capable of neutralizing streptolysin O and rendering it incapable of hemolyzing cells. This aspect of the streptolysin O and antistreptolysin reaction is made use of in the test we commonly call the antistreptolysin O test. In addition, we will discuss the screening test for streptococcal exoenzymes, Streptozyme.

438. Indicate whether given statements correctly reflect the procedure, reagents, and significance of ASO titration.

The ASO Titration. In the antistreptolysin test, you expose a serial dilution of suspected serum (inactivated or not) to a standardized streptolysin O solution. After a period of incubation, add human or rabbit red cells to each tube of the dilution. If the patient has developed antistreptolysin O antibodies, the standardized streptolysin O solution will be neutralized and the blood cells will *not* be hemolyzed. If no antibodies are present, the red blood cells will be hemolyzed by free streptolysin O.

Report the last dilution (tube) showing no hemolysis as the titer of the test. Test results are reported as Todd units (a titer of 100 equals 100 Todd units). Highest normal titers are seen in school age children. Their normal titers can be as high as 333 Todd units. Normal healthy adults usually do not have titers higher than 200 Todd units. This difference in normal values is primarily due to children having more streptococcal infections than adults.

Antistreptolysin titers are particularly high in rheumatic fever and glomerulonephritis. Most acute rheumatic fever patients have titers of 300 to 1500 Todd units. A small percentage may, however, exhibit normal ASO titers. The titers remain high for as much as 6 months in this disease. In suspected rheumatic fever and glomerulonephritis, tests are run in series several weeks apart. An increasing titer is more indicative of rheumatic fever than a single high titer.

An increased ASO titer can be seen in many infections and diseases. Included among these are chronic sore throat, severe acne, pneumococcal pneumonia, and rheumatoid arthritis. The titer seen in these diseases is not as high nor does it persist as long as the elevated titer in rheumatic fever. If the patient with

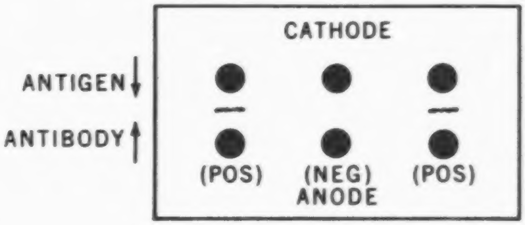

Figure 3-3. Immunoelectroosmophoresis (IEOP), example using Australia antigen.

rheumatic fever is being treated with antibiotics, such as penicillin and chlortetracycline, a decrease in titer may occur from the beginning of treatment. These antibiotics can inhibit the in vivo production of streptolysin O. This inhibition lessens the immune response, resulting in lowered titers.

Quality control of the ASO test does not differ from that of any other serological procedure. There are two common sources of error; guard against these to insure accurate tests. The first is to be sure to use fresh cells if possible. Wash these cells thoroughly to remove any residual antistreptolysin O antibodies that the donor may have possessed. The second thing to watch is the streptolysin O reagent used in the test. As stated before, streptolysin O is oxygen-labile. If left standing at room temperature or if reconstituted and put into the refrigerator, the streptolysin O reagent becomes inactivated. It must be used *immediately* after reconstitution. Improper handling of this reagent is the most common source of error in the ASO test.

Exercises (438):

Indicate whether each of the given statements is true (T) or false (F), and correct those that are false.

T F 1. In the antistreptolysin test, a serial dilution of suspected serum is exposed to a standardized streptolysin O solution.

T F 2. If the patient has developed streptolysin O antibodies, the standardized streptolysin O solution will not be neutralized and the blood cells will be hemolyzed.

T F 3. If no antibodies are present, the red blood cells will be hemolyzed by free streptolysin O.

T F 4. The last dilution showing no hemolysis is reported as the titer.

T F 5. A titer of 100 equals 200 Todd units.

T F 6. The difference in normal values is primarily due to children having more streptococcal infections than adults.

T F 7. An increasing titer is more indicative of rheumatic fever than a single high titer.

T F 8. An increasing ASO titer may be seen in chronic sore throat, severe acne, pneumococcal pneumonia, and rheumatoid arthritis.

T F 9. The donor's cells need not be washed since residual streptolysin O antibodies cannot affect the test.

T F 10. If left standing at room temperature or if reconstituted and put in the refrigerator, the streptolysin O reagent becomes inactivated.

439. Indicate whether given statements correctly reflect the reagents, significant features, and importance of the screening test for streptococcal exoenzymes.

Streptozyme Test for Streptococcal Exoenzymes. The Streptozyme test is a 2-minute slide hemagglutination procedure which quantitatively measures multiple antibodies to streptococcal extracellular products. The reagent consists of aldehyde-stabilized sheep erythrocytes sensitized with the extracellular products obtained from broth supernatants of group A streptococcal cultures. The sheep erythrocytes are sensitized simultaneously with streptolysin O, streptokinase, hyaluronidase, deoxyribonuclease (DNase), and nicotinamide adenine dinucleotidase (NADase). Agglutination of the erythrocytes indicates the presence of antibodies in the serum against one or more of the streptococcal exoenzymes.

Reagents and equipment are available commercially in kit form. If control sera give the expected results, report the results according to the manufacturer's instructions. Data have been presented which indicate that the Streptozyme test detects a much higher incidence of elevated antistreptococcal antibodies than the single ASO test. The specificity of the test, the reproducibility, the ease of performance, and the minimal quantity of patient serum required are significant considerations for the serologist when choosing an excellent diagnostic test. Currently, most clinical diagnostic laboratories perform only a single streptococcal antibody determination, the ASO test. Unfortunately, approximately 20 percent of the patients with acute rheumatic fever have normal serum titer of ASO. In addition, the ASO response to streptococcal cutaneous infection is weak and inconsistent. In areas of the United States and the world in which streptococcal pyoderma and pyoderma-associated acute poststreptococcal glomerulo nephritis are the principal streptococcal diseases, the ASO test is of very limited value. The Streptozyme test is not affected by hemolysis, increased cholesterol levels, or anticoagulants.

Positive tests may be titered by preparing further dilutions of serum in saline and testing each dilution as an individual serum would be; however, the additional cost involved probably makes the hemolytic ASO test more practical for titration of ASO antibodies. There are also tests available for the titration of antibodies against the individual streptococcal exoenzymes, other than ASO, but these tests are generally impractical for the average laboratory.

Whole blood may be used instead of serum if the dilution due to the erythrocytes is taken into consideration in making the 1:100 dilution.

This test may be positive due to a cumulative effect of the five possible antibodies that may be reacting with the coated erythrocytes. Titers of each of the individual antibodies might be below significant levels.

Although a true correlation cannot be made between the results of this test for five exoenzyme antibodies and the hemolytic ASO test, it has been found that the 1:100 dilution of serum is equivalent to a 1:166 dilution of serum (166 Todd units) in the hemolytic ASO test.

This test may be repeated at weekly or biweekly intervals to detect changes in titers.

Exercises (439):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. The Streptozyme test quantitatively measures multiple antibodies to streptococcal intracellular products.

T F 2. The reagent consists of aldehydestabilized sheep erythrocytes sensitized with products obtained from broth supernatants of group B and D streptococcal cultures.

T F 3. The sheep erythrocytes are also sensitized with streptococcus MG antibodies.

T F 4. Agglutination of the erythrocytes indicates the presence of antibodies in the serum against one or more of the streptococcal exoenzymes.

T F 5. Unfortunately, 20 percent of the patients with acute rheumatic fever have normal serum titer for Streptozyme.

T F 6. The ASO response to streptococcal cutaneous infection is weak and inconsistent.

T F 7. The Streptozyme test is not affected by hemolysis, increased cholesterol levels, or anticoagulants.

T F 8. It has been found that the 1:100 dilution of serum in the Streptozyme test is equivalent to a 1:100 dilution in the ASO test or 100 Todd units.

CHAPTER 4

Serological Tests for Syphilis

SYPHILIS IS A contagious venereal disease caused by the spirochete, *Treponema pallidum*. The usual site of invasion of the treponeme is the male's glans penis and female's cervix, but other parts of the genitalia may be the invasion site. Although the disease is contracted primarily during direct sexual contact, invasion may be through other mucous membranes, such as of the mouth or anal region. Even abrasions or cuts in the skin of other parts of the body may serve as sites of entry of the treponeme—a fact that makes syphilis an occupational hazard for members of the medical profession.

Another infection route—one that justifiably generates a considerable amount of laboratory work—is the transmission of treponemes from a syphilitic pregnant woman across the placental barrier to her developing fetus. The resulting disease is referred to as congenital syphilis. This condition may (and should) be treated in utero by treating the mother as soon as her infection is detected. Many of these cases result in abortions and stillbirths. However, some infants are born alive with signs of syphilis already present. Some syphilitic infants are born without overt signs of the disease, but disfigurement, blindness, deafness, and other complications may develop unless treatment is started as soon as possible.

Fortunately, the treatment of all types of syphilis, including congenital syphilis, is adequately performed with penicillin. Only rarely have cases failed to be completely cured by one or two doses of the drug. Therefore, the persistent (and increasing) incidence of syphilis is not due to inadequate therapy, but the disease persists due to changing sociological standards and—of prime interest to the serologist—frequent difficulties in diagnosing the disease. These diagnostic difficulties are compounded by the fact that untreated venereal syphilis may go through four distinct stages, but exact stages and their manifestations are not predictable from patient to patient.

In this chapter, we will recognize the two distinct forms of syphilis, related infections, and serological tests for syphilis.

4-1. Syphilis and the Serology of Syphilis

Many interesting things have been written about syphilis, partly because syphilis made an impact throughout modern history and also because this is a venereal disease with some rather frightening symptoms. It leads to infiltration and destruction of brain tissue, and this results in disability, insanity, and death. Evidence suggests that syphilis first appeared about 500 years ago, though medical practices and methods of reporting may have obscured the prevalence of the disease at earlier times in history. Many historians believe that syphilis was first introduced into Europe by members of Columbus' crew when they returned to Europe from the West Indies. Incidentally, it is suggested that Columbus himself may have suffered from syphilis. During his third voyage in 1498, Columbus was described as having a "severe attack of gout." He developed mental symptoms and finally, after his last voyage in 1504, had to be carried ashore. Columbus had all the symptoms of terminal syphilis.

In 1496 syphilis spread throughout Europe and was known as "The Great Pox." Affected individuals were quarantined and in many cases banished to islands or colonies similar to leper colonies. By the early 1500s syphilis had spread to England and eastward to China. You may recall from popular accounts that Italians called syphilis the French or Spanish disease, the English called it the French disease, and the French called it the Spanish disease.

The term *syphilis* was coined by Fracastorius, the famous Italian physician, when he wrote a poem about the disease in 1530. Syphilis was a shepherd in the poem who was stricken with this dreadful disease by Apollo. Perhaps Fracastorius contributed to the prevalent belief that syphilis represented a punishment for sin and was not to be approached with quite the same "objectivity" as nonvenereal diseases.

Today, two forms of syphilis are recognized. The first is *venereal syphilis* and is worldwide in occurrence. ("Venereal" relates to sexual intercourse.) The second type of syphilis is *endemic syphilis* and is confined to certain parts of the world outside of the United States.

440. Indicate whether given statements correctly reflect the stages and characteristics of untreated venereal syphilis.

Venereal Syphilis. Venereal syphilis is characterized clinically by a primary lesion, a secondary eruption involving the skin and mucous membranes, and finally after what is often a long latent period, lesions of the skin, internal organs, bones, and central nervous system.

Primary syphilis. The first lesion, often called the *primary* lesion or chancre appears abot 3 weeks after infection. Needless to say, diagnosis is left to a physician, particularly since other disease processes may involve a lesion and also because treatment is necessary. Diagnosis of primary syphilis is accomplished mainly by darkfield microscopic examination from the chancre for treponemes. Although a small percentage of cases have detectable levels of antibodies during the primary stage of the disease, a nonreactive serologic test for syphilis at this time does not rule out the disease.

Usually, after the apparent spontaneous resolution of the primary chancre in untreated syphilis, there is a period when no visible signs of the disease are present. The serologic tests are nearly always reactive during this period, which may last up to several months. Occasionally, however, the secondary stage of syphilis may start before the primary lesion has disappeared. One sometimes hears of individuals, often medics, who are not physicians, who attempt to treat themselves or their acquaintances to avoid possible administrative or social consequences. This can be dangerous becuase inadequate treatment in the primary stage will mask symptoms and lead to the more serious secondary stage.

*Secondary syphilis.*This stage of syphilis usually starts before or within a few months after the disappearance of the primary lesion. It is characterized by fever, malaise, mucous patches in the mouth, condylomata of the ano-genital region, and a localized or generalized rash. All of these lesions are highly infectious. In some patients, these lesions last only a few days, but they may last up to 1 year. Periodic recurrences of these signs and symptoms may occur for a couple of years.

Diagnosis of secondary syphilis may be made by darkfield examination of the lesions. In addition, nearly 100 percent of patients in this stage will have reactive serologic tests on serum.

With treatment, the symptoms of secondary syphilis disappear rapidly. Even without treatment, symptoms eventually disappear, and the disease usually enters a latent period unless the disease progresses directly into the tertiary stage of syphilis.

Latent syphilis. This stage of the disease is characterized by reactive serologic tests for syphilis on serum, but the cerebrospinal fluid (CSF) must be nonreactive. If the CSF is reactive, the disease has already entered the tertiary stage. No other manifestations of syphilis are present during the latent stage which may last several years or for the life of the patient. An additional feature of latent syphilis is that the disease is no longer communicable to another individual EXCEPT transplacentally to a fetus.

Tertiary(late) syphilis. The manifestations of tertiary syphilis are varied and generally do not appear for several years after the primary infection. Cases may resemble an apparent latent stage for up to 40 years before symptoms appear. Tertiary syphilis may involve the central nervous system with the possibility of insanity. The cardiovascular system is also frequently affected, with the most serious complication being the development of an aortic aneurysm which may rupture and result in nearly instantaneous death. In addition, lesions may be produced in the skin, bone, and internal organs. These lesions are not infectious, EXCEPT that a developing fetus may become infected even during this late stage of the disease. All of these serious complications of syphilis make the early diagnosis and treatment of syphilis a professional challenge. Fortunately, most cases of tertiary syphilis, as well as secondary and latent syphilis, will have reactive serologic test for syphilis. In addition, the spinal fluid of cases with central nervous system involvement will usually have reactive tests.

As you know, the infectious agent is *Treponema pallidum*, a spirochete. Man is the only reservoir, and infection is by means of exudates from lesious of the skin or mucous membranes. Indirect contact is considered of little significance because *Treponema pallidum* does not survive under conditions of unfavorable moisture, temperature, and the like. It is possible to contract the disease while handling body fluids in the laboratory where such fluids are taken from areas of active early lesions. Serum is not *usually* considered a potential source of infection to the laboratory technician, but it could be. Prenatal infection may occur after the fourth month of pregnancy through placental transfer. Transmission of syphilis by blood transfusion is unlikely because of the storage temperature of blood and the survival time of *Treponema pallidum* under storage conditions. However, as you know from your reading earlier in this course, blood donors are screened to preclude the use of blood from a donor with syphilis.

There is no natural immunity to either venereal syphilis or endemic syphilis. Infection does lead to a gradually developing resistance to strains which are closely related, but there is little protection afforded to heterologous strains. The control of syphilis from an epidemiology standpoint is a matter for the Environmental Health Services of the hospital, and this involves the laboratory only indirectly. Often, coordination of laboratory data such as positive serological tests may be of value to the hospital Registrar or Environmental Health Services officials. You must be careful in drawing conclusions from laboratory data alone, however.

Exercises (440):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. The primary lesion or chancre appears about three days after infection.

T F 2. Diagnosis of primary syphilis is accomplished mainly by a reactive serologic test.

T F 3. A nonreactive test for syphilis in the primary stage rules out the disease.

T F 4. A medic who treats himself or his acquaintances for syphilis to avoid possible administrative or social consequences has made the correct decision.

T F 5. Diagnosis of secondary syphilis may be made by darkfield examinations of the lesion, and nearly 100 percent of patients in this stage will have reactive serologic tests on serum.

T F 6. In the latent stage, the symptoms have eventually disappeared, it is characterized by reactive serologic tests for syphilis on serum, and the cerebrospinal fluid (CSF) must be reactive.

T F 7. The secondary stage has no other manifestations of syphilis and may last several years or for the life of the patient.

T F 8. An additional feature of secondary syphilis is that the disease is no longer communicable to another individual, except transplacentally to a fetus.

T F 9. Tertiary syphilis may involve the central nervous system with the possibility of insanity, blindness, and other neurological problems.

T F 10. Most cases of tertiary syphilis as well as secondary and latent syphilis will have reactive serologic tests for syphilis.

T F 11. Man is the only reservoir, and infection of the organism is by means of exudates from lesions of the skin or mucous membrane.

T F 12. It is not possible to contract the disease while handling body fluids in the laboratory, although such fluids may be taken from active early lesions.

T F 13. Transmission of syphilis by blood transfusion is very likely because *Treponema pallidum* survives ideally under the storage temperature of blood.

T F 14. There is no natural immunity to either venereal or endemic syphilis.

T F 15. The control of syphilis from an epidemiology standpoint is a matter for the entire laboratory service.

T F 16. A syphilis infection elicits a stable or long-lasting immune response.

441. State the difference between endemic and venereal syphilis and point out three serologically-related diseases that give false positive syphilis tests.

Endemic or Nonvenereal Syphilis. This type of syphilis is acute and of limited geographical distribution. It is transmitted by direct and indirect contact with infectious lesions, often spread by the use of common eating and drinking utensils. The infectious agent is *Treponema pallidum*. Thus, you see that unless a strain different from that which causes venereal syphilis is involved, endemic syphilis is etiologically the same as venereal syphilis. But from the standpoint of the mode of transmission and geographical occurrence, the distinction between venereal and nonvenereal syphilis is valid and useful, especially in efforts to control communicable diseases. Further, unlike venereal disease, nervous and cardiovascular system involvement is rare with endemic syphilis. Fatalities are also rare in cases of nonvenereal syphilis. It is common in Africa, the Balkans, and along the Mediterranean. This disease also has various synonyms, including *bejel, dichuchwa, njovera, sibbens,* and *radesyke*.

Serologically Related Infections. There are several spirochete diseases which give false results in serological tests used to detect the syphilis antibody. Among these diseases are yaws, pinta, and rat-bite fever. In addition, positive serological tests have been reported in a variety of etiologically unrelated disorders, including disseminated lupus erythematosis.

Yaws. One of the diseases which presents a serological picture easily confused with that of *T. pallidum* is *yaws*, an acute and chronic nonvenereal tropical and subtropical disease caused by *Treponema pertunue*. Yaws resembles syphilis but does not involve the viscera or central nervous system. Neither does it occur congenitally. (Congenital transmission of *nonvenereal* syphilis is described as "rare.") Yaws is predominantly a childhood disease but often does occur in older people, especially males. It is common in Southeast Asia, in Africa, the Philippines, throughout the South Pacific Islands, in the Caribbean, and in South America. Serological tests for syphilis present a confusing picture in these areas because of the incidence of antibodies to *T. pertenue*. Like syphilis, there is no evidence of natural or racial resistance, except that infection results in immunity to homologous strains.

Pinta. This is an acute as well as a chronic infection by *Treponema carateum* which causes some of the serological tests for syphilis to give a positive reaction. Serological tests for syphilis usually become reactive during the secondary rash of this disease. It is a common disorder in the tropics and subtropics but is not limited to these areas. It is also common in Mexico and certain countries of South America, especially Venezuela, Colombia, and Ecuador. In addition, pinta is found in North Africa, the Middle East, India, and the Philippines.

Rat-bite fever. The term *rat-bite fever* is nonspecific. It refers to two different diseases transmitted by bites of infected rats. One of the diseases is known as *Streptobacillus moniliformis* disease and the other as *Spirillum minus* disease. It is the latter which presents a syphilis-like serological pattern and, to some extent, a clinical picture similar to syphilis. The disease is sometimes called sodoku, or sporadic rat-bite fever. The correct name of the organism that causes *Spirillum minus* disease is *Spirocheta morsus muris.* It is common in Japan and in the Far East. The important thing for you to realize is that there are many relatively common diseases, including the three just described (yaws, pinta, and rat-bite fever) which cause positive serological tests for syphilis.

Exercises (441):

1. How does transmission of endemic syphilis differ from venereal syphilis?

2. How does endemic syphilis differ from venereal syphilis in terms of nervous and cardiovascular system involvement? Fatalities?

3. Name three serologically related diseases that give false positive syphilis tests.

4. What organism causes yaws?

5. How does yaws differ from syphilis?

6. The organism *Treponema carateum* causes what syphilis-like disease?

7. What are the two organisms that cause rat-bite fever?

4-2. Types of Serologic Tests for Syphilis

The serodiagnosis of syphilis is based on the detection of two broad categories of antibodies. Most of the tests (especially the screening tests) are based on the detection of antibodies against antigens that have no logical relationship to the causative treponemes. These tests are referred to as nontreponemal or reagin tests. In addition, several tests have been developed that use antigens that are treponemal in origin. Examples of both types will be presented in this section.

442. Cite the two basic types of serologic tests for syphilis and specify the principle, the reagents, and the usefulness of each technique used with both types of tests.

Nontreponemal (Reagin) Tests. The antigen used in most of these tests is an extract of beef heart, known as cardiolipin. The cardiolipin may be mixed with lecithin and cholesterol for use in microscopic flocculation tests such as the Venereal Disease Research Laboratory (VDRL) and the Untreated Serum Reagin (USR) tests. Cardiolipin has also been combined with carbon particles (charcoal) to produce macroscopic tests, such as the RPR tests. In addition, various nontreponemal antigens have been used in complement fixation as well as several other varieties of flocculation tests. These nontreponemal tests are usually referred to collectively as a "blood test," "serology," or, more specifically, "STS," which translates to "serologic test for syphilis." These tests are also frequently called a "VDRL," but "VDRL" actually refers to one particular reagin test.

The syphilitic antibodies against nontreponemal antigens are considered to be antibody-like because the antigens in use cannot stimulate their production, so they are not true antibodies. They are also called reagins or reaginic antibodies to differentiate them from treponemal antibodies.

Because nontreponemal (reaginic) antibody production is not specifically stimulated by the treponemes, several other conditions can cause these antibodies to be produced. Malaria, leprosy, hepatitis, and certain immunizations are just a few of the conditions that can cause reactive nontreponemal tests at varying rates. These results are called biological false positives—BFP, for short. These BFPs, along with the social implications of syphilis, are the reason that serologic tests for syphilis are reported as "Reactive, Weakly Reactive, or Nonreactive" and NOT "Positive" or "Negative." These conditions causing BFPs must be differentiated from actual treponemal infections. Reactive tests due to related treponemal infections, such as bejel, pinta, and yaws, should not be classified as BFPs. Fortunately, tests on spinal fluid rarely produce BFPs; a reaction usually indicates syphilis—in fact, tertiary syphilis.

In addition to their usefulness as screening tests for syphilis, nontreponemal (reagin) tests are helpful in following the effectiveness of therapy. The antibody titer in these tests drops rapidly following successful treatment of primary syphilis. In secondary syphilis, the titer drops more slowly but usually will eventually become nonreactive. Unfortunately, titers in treated latent and tertiary syphilis may remain fairly constant for years or life. By contrast, titers of the tests using treponemal antigens do not normally drop; therefore, these reagin tests are at least useful in following treatment of the early stages of syphilis.

Treponemal Tests. Serologic tests for syphilis that utilize the treponeme as the source of antigen generally require more sophisticated equipment and techniques that reagin tests. However, the main advantage of treponemal tests, such as the Fluorescent Treponemal Antibody-Absorption Test (FTA-ABS), is that biological false positives are essentially eliminated. In fact, the FTA-ABS test is frequently used to confirm or reject the findings of one of the nontreponemal tests when these results are questionable. Although the FTA-ABS test is currently the most widely used treponemal-type test, two other tests are still in use, but declining. These are the Treponema Pallidum Immobilization Test (TPI) and the Reiter-Protein Complement Fixation Test (RPCF). Only the FTA-ABS will be presented in detail in this section.

Exercises (442):

1. What are the two types of serologic tests used for syphilis?

2. What are the constituents in the antigen used in microflocculation tests such as the VDRL and USR tests?

3. In the RPR test, cardiolipin is combined with what substance so as to form the antigen?

4. The syphilitic antibodies against nontreponemal antigens are considered to be antibody-like and are called ______________ or ________ ________.

5. What are some conditions that can cause the production of reaginic antibodies?

6. Conditions that cause reactive nontreponemal tests produce results called __________ ___________ _____________________ or _______________.

7. How are serological tests for syphilis reported?

8. Reactive tests, due to related treponemal infections such as bejel, pinta, and yaws, (should be/should not be) classified as BFPs.

9. In addition to their usefulness as screening tests, what other purpose do nontreponemal (reagin) tests serve?

10. What is the main advantage in the use of treponemal tests?

11. Name three treponemal tests.

443. Specify the purpose for reagents and steps used in the qualitative and quantitative VDRL (Cardiolipin Microflocculation slide test, the guidelines for interpretation and reporting of results, and cite some sources of error.

Qualitative VDRL (Cardiolipin Microflocculation) Slide Test. Cardiolipin antigen (160 ml) containing lecithin and cholesterol is added to 0.05 ml of inactivated serum on a slide with 14 mm paraffin or ceramic rings. The slide is rotated for 4 minutes at 180 rpm. After rotation, the mixture is examined microscopically at 100X magnification for flocculation of the antigen particles. Flocculation indicates the presence of reaginic antibodies in the serum. The antigen used in this test is composed of cardiolipids and lecithin which have been extracted from beef heart and purified. Cholesterol is added to the alcoholic mixture of the cardiolipids and lecithin for the purpose of increasing the antigen's effective reacting surface. The antigen and buffered diluent are supplied through Air Force supply channels; pH range of the diluent must be between 5.9 and 6.1. The qualitative and quantitative VDRL procedures are described in AFM 160–47, *Clinical Laboratory Procedures—Serology*.

Inactivate clear sera at 56° C for 30 minutes. Sera, such as control sera, that were inactivated more than 4 hours before being tested and sera to be tested must be reheated for 10 minutes at 56° C. Cool all sera to room temperature before being tested.

Before any results can be reported, the control sera must produce the expected results. If the control sera indicate valid results, report the patient's sera according to reactions specified in AFM 160–47, preferably along with the titer of all Reactive, Weakly Reactive, or "rough" sera. If any reaction is observed in the qualitative test, the serum should be serially diluted and checked in the quantitative test.

Quantitative CMF. The quantitative cardiolipin microflocculation test is performed on all specimens yielding weakly reactive or reactive results in the qualitative test. The reagents and apparatus employed in the qualitative test are used in the quantitative procedure as well. All of the preliminary testing should also precede the quantitative procedure. Quantitative tests are performed with serial dilutions of serum in normal saline. Each dilution is treated as an individual serum. The highest dilution of serum producing a Reactive (not Weakly Reactive) result is reported as the titer.

Interpretation of results. A reactive or weakly reactive test result indicates the presence of reagin, which almost invariably is formed in treponemal infection, but which may be produced by a variety of other conditions. Medical practice considers a reactive result in the presence of clinical symptoms as confirmatory evidence of syphilitic infection. However, in the absence of clinical findings, test reactivity can represent any of the following: latent syphilis; a biological false positive reaction, either temporary or chronic; or a technical or clinical error. The simplest step open to the physician is to request VDRL testing on a repeat specimen. The titer on the second specimen can then be compared with that of the first; a drop to nonreactive suggests a prior technical problem or a temporary biological false positive condition in the patient; a rise in titer suggests the likelihood of syphilis; a stable titer remains inconclusive, requiring further medical follow-up as well as serological testing for treponemal antibodies. On the average, reagin does not become demonstrable until 6 to 8 weeks after infection. Occasionally the CMF tests for syphilis become positive by the fourth or fifth week following infection with *Treponema pallidum*. At the other extreme, in occasional instances, reagin may not become demonstrable in the blood until 12 weeks after infection.

Once reagin begins to form, it usually increases rapidly until it reaches a peak during the secondary stage. After the secondary stage has ended, as a rule the amount of reagin in the blood decreases until it reaches a fairly stable level during the late course of the disease.

Sources of error. There are a number of possible sources of error inherent in CMF tests. Some techniques for preventing many of these errors are listed here.

a. All reagents must be at room temperature for testing. Cold reagents may decrease test sensitivity; if they are too warm, the test may be hypersensitive.

b. This test must be performed only on clear serum. Cloudy serum or heated plasmas may contain confusing precipitates.

c. Biological false positive are possible.

d. Prozonal reactions may occur; therefore, all Weakly Reactive, as well as "rough" reactions, should be titered to detect potentially high titered sera.

e. Mix the antigen emulsions frequently to insure an even suspension of antigen particles.

f. The pH of the buffered saline is critical. Alterations may affect test sensitivity.

g. The antigen emulsion should only be used on the day it is prepared.

Exercises (443):

1. In the qualitative cardiolipin microflocculation test, what does flocculation indicate?

2. For what purpose is cholesterol added to the alcoholic mixture of cardiolipids and lecithin?

3. Sera, such as control sera and sera to be tested, that were inactivated more than 4 hours before being tested must be reheated for ________ minutes at ________ .

4. In the quantitative CMF test, what should the reaction of the highest dilution of serum be to be reported as the titer?

5. When there is a drop to a nonreactive in the titer on the second specimen, what possible conditions exist?

6. On the average, how long after the infection does reagin become demonstrable?

7. After the secondary stage has ended, as a rule, what happens to the level of reagin in the blood?

8. What type of error may be caused by cloudy serum or heated plasmas?

9. Why should the antigen emulsions be mixed frequently?

444. Briefly state the procedure for the Rapid Plasma Reagin (RPR) test, the manner of reporting results, and some possible sources of error.

Rapid Plasma Reagin (RPR) Test. Many laboratories are now using the RPR card test *as a screening procedure*. The emulsion is a carbon-containing, specially formulated cardiolipin antigen, and the flocculation is observed on plastic coated cards. The RPR antigen should be checked with controls prior to each series of tests. The entire test kit, including controls, is available commercially. Instructions and precautions are supplied by the manufacturer. In the test, unheated serum (0.05 ml) is spread within an 18 mm circle on a plastic coated card. A drop (1/60 ml) of carbon-containing RPR antigen is added to the serum without mixing. The card is rotated on a slide rotator circumscribing a 3/4-inch circle for 8 minutes at 100 rpm. Macroscopic flocculation of the carbon particles indicates the presence of reaginic antibodies in the serum.

Reagents and equipment. Reagents and equipment should include the following:

a. Patient's serum, unheated.

b. control sera of known reactivity, available commercially or collected locally.

c. RPR card test kit, 18 mm circle, available through federal supply channels complete with cards, capillaries for dispensing serum, and antigen suspension with a dispensing needle. NOTE: Several other types of RPR kits are available from Hynson, Westcott, and Dunning. These kits, including one for hand rotation, may more adequately fulfill the objectives of a given laboratory. Be sure to follow all instructions for the test selected.

d. Slide rotator, circumscribing a 3/4-inch circle and set at 100 rpm.

e. Humidifying cover with moist blotter to cover cards during rotation.

Reporting results. If the control sera produce the expected results, report the patient's sera as follows:

(1) Sera producing no flocculation of the carbon or only a slight roughness—Nonreactive.

(2) Sera producing flocculation (clumping) of the carbon particles—Reactive, preferably along with a titer. Procedures for a quantitative card test are included with the manufacturer's instructions.

Sources of error. There are a few possible sources of error in the RPR test. The following techniques for preventing these errors are listed here.

a. Failure to follow manufacturer's instructions, especially storage instructions and expiration dates of the antigen, may produce invalid results.

b. Scratched cards may resemble flocculation.

c. All reagents must be at room temperature for testing. Cold reagents may decrease test sensitivity—if they are too warm, the test may be hypersensitive.

d. Dispensing needles must be accurate. Each serologic test may use a different size of antigen drop, so don't interchange needles without testing them. Before storing the needle, be sure to blot all fluid from it, because dried antigen may alter the drop size, but do not wipe the needle since this removes the silicone coating.

e. Do not confuse the 18 mm circle test with the 14 mm card test which uses different volumes of antigen and plasma. Still other tests for syphilis use 14 mm ceramic or paraffin rings on glass slides.

f. Biological false positive results are possible with this test.

g. Plasma collected with a variety of anticoagulants may be substituted for serum. Check the manufacturer's instructions for acceptable anticoagulants.

Exercises (444):

Complete each sentence with the appropriate word or words. A phrase rather than a single word may be required for the correct response.

1. In the RPR test, __________ ml of unheated serum is spread within an __________ mm circle on a plastic card.
2. A drop __________ ml of __________ is added to the serum without mixing.
3. The card is rotated on a slide rotator for ___ minutes.
4. In order to prevent drying during rotation, a __________ __________ with moist blotter is used to cover the cards.
5. Sera producing flocculation are reported as __________ preferably along with a __________ __________.
6. Cold reagents may __________ test sensitivity, and reagents that are too warm may __________ test sensitivity.
7. Before storing the needle, be sure to __________ all fluid from it.
8. Dried antigen in the needle may __________ the drop size.
9. Biological false positive results are (possible/not possible) with this test.
10. __________ collected with a variety of anticoagulants may be substituted for serum.

445. Indicate whether given statements correctly reflect the procedure for the VDRL slide test on spinal fluid, guidelines for reporting results, and sources of error; identify the uses of the colloidal gold test and the FTA-CSF tests.

VDRL Slide Test on Spinal Fluid. VDRL slide test antigen is made more sensitive by adding an equal volume of 10-percent saline. A drop (1/100 ml) of this sensitized antigen is added to 0.05 ml of unheated spinal fluid in a 16 mm diameter by 1.75 mm deep concavity of an agglutination slide. The slide is rotated for 8 minutes at 180 rpm. After rotation, the mixture is examined microscopically at 100X magnification for flocculation of the antigen particles. Flocculation indicates the presence of reaginic antibodies in the spinal fluid.

Reporting results. Before any results can be reported, the control sera must produce the expected reactions. If the controls indicate valid results, report the patient's spinal fluid as either Reactive or Nonreactive, as appropriate. NOTE: The Weakly Reactive report is not used for this test. Any reactivity is significant.

Sources of error.

a. Be sure that the VDRL antigen emulsion is sensitized by adding equal parts of 10 percent saline.

b. The antigen drop (1/100 ml) must be accurate within ± 2 drops/milliliter.

c. Reactive control sera must have a titer of at least 1:80 when tested by the spinal fluid test.

d. Spinal fluids containing gross blood or microbial contamination are unsatisfactory.

e. All reagents must be at room temperature for testing.

f. False positives rarely occur in tests for syphilis performed on spinal fluid. A Reactive test usually indicates present or past neurosyphilis, especially if the cell count and protein content of the spinal fluid are elevated.

g. The spinal fluid must be Nonreactive in latent syphilis. A Reactive spinal fluid indicates that the disease has progressed to the tertiary stage.

h. The spinal fluid is Reactive in about one-fourth of cases in the secondary stage of syphilis, thus indicating that central nervous system involvement has begun. The VDRL procedure is outlined in AFM 160-47.

Colloidal Gold Test on Spinal Fluid. The colloidal gold test on spinal fluid was widely used in the past for the differentiation of the various types of neurosyphilis and other central nervous system conditions. In the test, a colloidal suspension of gold is added to serially diluted spinal fluid. Abnormal spinal fluid proteins cause partial to complete precipitatin of the gold with a variety of color changes. More recently this test was mainly used in the study of multiple sclerosis, but the test is being rapidly replaced by the simpler, and more reproducible, gum mastic and benzoin tests.

FTA-CSF Test. A new procedure, the Fluorescent Treponemal Antibody Test for Cerebrospinal Fluid (FTA-CSF), is undergoing evaluation and thus is not completely ready for general use. In the procedure, undiluted or diluted cerebrospinal fluid, producing the most optimal results, is placed on top of the treponeme and the tests carried out in the same way as for serum. The test is highly sensitive for *Treponema pallidum* antibodies in the spinal fluid. The only problem is deciding the significance of such antibodies. This question is the subject of several ongoing clinical studies.

Exercises (445):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. VDRL slide test antigen is made more sensitive by adding an equal volume of 1 percent saline.

T F 2. A drop (1/100 ml) of the sensitized antigen is added to 0.5 ml of unheated spinal fluid in a 16 mm diameter by 1.75 mm deep concavity of an agglutination slide.

T F 3. After rotation for 8 minutes at 180 rpm, the slide is examined macroscopically for agglutination.

T F 4. The Weakly Reactive report is not used when reporting VDRL of spinal fluid.

T F 5. Be sure the BDRL antigen is sensitized by adding equal parts of 1 percent saline.

T F 6. Spinal fluids containing gross blood or microbial contamination do not affect the test results.

T F 7. False positives are rare in tests for syphilis performed on spinal fluid.

T F 8. A Reactive test usually indicates present or past neurosyphilis, especially if the cell count and protein content of the CSF are elevated.

T F 9. The spinal fluid must be reactive in latent syphilis.

T F 10. The colloidal gold test on spinal fluid was widely used in the past for the differentiation of the various types of neurosyphilis and other central system conditions.

T F 11. More recently, the colloidal gold test was mainly used in the study of multiple sclerosis, but it is rapidly being replaced by gum mastic and benzoin tests.

T F 12. The fluorescent treponemal antibody test for cerebrospinal fluid (FTA-CSF), a new procedure, is highly sensitive for *Treponema pallidum* antibodies.

446. Identify given treponemal antigen tests in terms of the principles, reagents, applications, and sources of error.

Reiter Protein Complement Fixation (RPCF) Test. This test is the Kolmer complement fixation method using an extract of the Reiter strain of treponeme as the antigen. In the test, complement is "fixed" during the reaction of the Reiter protein and treponemal antibodies in the syphilitic patient's serum. Complement is, therefore, no longer available when the second antigen-antibody system, sheep erythrocytes and hemolysni, is added—hemolysis cannot occur. The complexity and relative insensitivity of the test originally led to the test being essentially replaced by the TPI test described in the next paragraph. More recently, the FTA-ABS test has replaced nearly all other treponemal tests for syphilis. This test is discussd here for historical interest. It has proved to be less sensitive than desirable, however, and has gradually faded from use in the United States.

Treponema Pallidum Immobilization (TPI) Test. The TPI test detects treponemal antibodies that immobilize living Nichols strain of *T. pallidum* in the presence of complement. The requirement for live treponemes from rabbit testes severely limits the practicality of this test for the average laboratory. Although the test is highly specific for treponemal diseases, it has been essentially replaced by the FTA-ABS test. Unlike the FTA-ABS test, the TPI test frequently becomes negative following treatment of the disease. The major problem with the TPI test is that it is very cumbersome. To obtain the antigen suspension of living treponemes, the animals are sacrificed and their tissue extracted. Thus, overall, it is an expensive and time-consuming procedure. Fewer laboratories are performing this test. The test is difficult to quantitate because every serum dilution would have to be tested as a separate specimen in a test.

Automated Reagin Tests. Attempts to automate tests for syphilis have mainly involved the use of the RPR carbon-containing antigens and various AutoAnalyzer components (Technicon Instruments Corp.). Most recent applications have involved continuous filtering of the reactants on a moving strip of paper with visual interpretation of the flocculated carbon particles. Test speeds of up to 100/hr have been reported with results equivalent to manually performed tests.

Fluorescent Treponemal Antibody Absorption (FTA-ABS) Test. Earlier in this volume you reviewed some of the principles of fluorescent antibody testing. We will now discuss the FTA-ABS test.

In the FTA-ABS test, the patient's serum is first absorbed with an extract of Reiter treponemes to adsorb antibodies that are common to both saprophytic and pathogenic treponemes. The adsorbed serum is then added to the Nichols strain of *Treponema pallidum* that has been dried on the slide and fixed with acetone. After incubation at 37° C, excess serum is rinsed from the slide with phosphate buffered saline (PBS). Then, fluorescein-labeled anti-human globulin (conjugate) is added to the *T. pallidum* smear, and the slides are incubated again at 37° C. At the end of this incubation period, excess conjugate is rinsed from the slide with PBS. The slides are coverslipped wth a glycerine mounting medium and examined by fluorescent microscopy using the appropriate exciter and barrier filters. The presence of treponemal antibodies is indicated by a greenish fluorescence of the treponemes. The observations are compared with the fluorescence due to known control sera.

False positive reactions have been reported in cases of lupus erythematosus (L.E.) in this test. Many of these demonstrate a peculiar "beaded" fluorescence of the treponemes. Other false positives may be detected as the test is studied more.

Other treponemal diseases (pinta, bejel, and yaws) should be expected to give reactive results.

FTA-ABS antibodies usually appear in the serum during the primary stage of syphilis and persist for life.

Even after treatment the test usually remains reactive for life while most other serologic tests for syphilis become nonreactive, especially if treatment occurs before the tertiary stage of the disease.

The FTA-ABS test is especially useful in the diagnosis of tertiary syphilis because the reagin tests may be nonreactive in about one-fourth of these cases.

More recently other adaptations of the procedure have been evolved for special purposes. One is the FTA-ABS (IgM) test for syphilis in babies. Preliminary evaluations have shown this to be highly sensitive and specific. The procedure is performed exactly the same as the conventional FTA-ABS test except that a fluorescent antiserum monospecific for immunoglobulin M, or IgM, is used in the last step of the procedure.

Exercises (446):

Match each of the following treponemal antigen tests in column B with the statements in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

___ 1. In this test when the second antigen-antibody system—sheep erythrocytes and hemolysin—is added, hemolysis cannot occur.

___ 2. This test detects treponemal antibodies that immobilize living Nichols strain of *T. pallidum* in the presence of complement.

___ 3. The requirement for live treponemes from rabbit testes severely limits the practicality of this test for the average laboratory.

___ 4. Most recent applications have involved continuous filtering of the reactants on a moving strip of paper with visual interpretation of the flocculated carbon particles.

___ 5. Using this method, test speeds of up to 100/hr have been reported with results equivalent to manually performed tests.

___ 6. The patient's serum is first absorbed with an extract of Reiter treponemes to absorb antibodies that are common to both saprophytic and pathogenic treponemes.

___ 7. False positive reactions by this test have been reported in cases of lupus erythematosus (L.E.).

___ 8. Antibodies detected by this test usually appear in the serum during the primary stage of syphilis and persist for life.

___ 9. Since the reagin tests may be nonreactive in about one-fourth of these cases, this test is especially useful in the diagnosis of tertiary syphilis.

___ 10. The test detects specific IgM anti-*T. pallidum* antibodies which circulate around in the serum of a newborn infant actively infected with syphilis.

___ 11. This procedure is performed exactly the same as the conventional FTA-ABS except that fluorescent antiserum monospecific for IgM is used in the last step.

Column B

a. FTA-CSF test.
b. Fluorescent Treponemal Antibody Absorption (FTA-ABS) test.
c. Reiter Protein Complement Fixation (RPCF) test.
d. Automated Reagin test.
e. Treponema Pallidum Immobilization (TPI) test.
f. FTA-ABS (IgM) test.

4-3. Quality Control in the Serology Laboratory

A monitoring system for detecting variations which exceed the acceptable limits set for all steps in laboratory procedures is not unique in the serology laboratory. Quality control, applied here, signifies quality assurance and quality performance. This may encompass consideration of reagents, glassware and equipment used, technician error, environmental influences, or even such questions as personnel qualifications in a particular section of serology, laboratory space and facilities, record keeping, and labeling. The more significant areas will be discussed in this section.

447. Indicate whether given statements correctly reflect guidelines for quality control in the serology laboratory in terms of the environment, equipment, glassware, record keeping, and reagents.

Environment. One of the prime concerns of a quality control program in serology is the environment in which technicians are expected to perform adequate work.

Even though technicians are highly qualified and thoroughly trained, there are a number of environmental factors which can increase the probability of technician error. If there is inadequate space available and technicians are cramped, there is often too much unnecessary setting up and taking down of equipment for error-free conditions. If the ambient temperature is not controlled and technicians are too hot or too cold, errors invariably occur. In addition, much equipment operates optimally under controlled temperature conditions. A high level of noise allowed to exist in the laboratory can be distracting and may result in errors that would not occur under ideal conditions. Lighting should be so arranged that the thousands of close visual observations taken daily can be made without strain. Other conditions which can be distracting and contribute to technician error are patients or visitors walking through the laboratory, loud radios, gossiping, or even miniskirts.

Equipment. Equipment designed to give quantitative measurements must be checked daily prior to use with specimens or "controls" of known concentration to ensure that it is in good working condition. Centrifuges must be checked with tachometers; pH meters must be precalibrated with buffered standards; spectrophotometers must be calibrated with known standards. Temperature controlling equipment such as refrigerators, water baths, and incubators must be checked often with precalibrated thermometers. All of these measurements and calibrations exemplify that part of the quality control program related to equipment in the laboratory.

Glassware. Although disposable glassware items have eliminated or reduced problems associated with detergent residue on many pieces of laboratory glassware, quality control demands that good principles of glassware cleaning be employed for those items that are repeatedly used. Samples of glassware should be spot-checked for residual detergent with brom cresol green indicator; if detergent residue is present, the indicator will turn blue. Badly etched glassware should be replaced.

Recordkeeping. Inadequate records obviously can lead to misconceptions in identification of problems that develop insidiously in the laboratory or to errors in reporting end results. Labeling of all specimens and other materials is a form of recordkeeping which, when completely and properly carried out, will contribute immensely to the smooth operation of a quality program. As a minimum, properly completed labels should identify the material; state its specific characteristics, for example, its pH, molarity, ionic strength: give the date it was prepared; precautions for its use; and include the initials of the person who prepared it.

Reagents. As commercial reagents are employed, the manufacturer's instructions regarding use, storage, and outdating must be carefully followed.

Exercises (447):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. Technicians who are highly qualified and thoroughly trained are not affected by environmental factors that can increase the probability of errors.

T F 2. Inadequate space available for technicians can lead to conditions resulting in errors.

T F 3. If ambient temperature is not controlled and technicians are too hot or too cold, errors can occur.

T F 4. Technicians are not distracted by high level noise; thus, the tests performed need not be affected.

T F 5. Equipment designed to give quantitative measurements must be checked weekly prior to use with specimens or controls of known concentration.

T F 6. Badly etched glassware should be used only as a last resort or when no better glassware is available.

T F 7. Brom cresol green may be used as an indicator to spot-check residual detergent on glassware.

T F 8. Even though you made a batch of reagents, the initials of the department chief, rather than yours, should be written on the label.

T F 9. Inadequate record keeping can lead to errors in reporting out results.

T F 10. Even though the date on a commercially prepared reagent has expired, one day of use beyond this date is permissible.

448. Indicate whether given statements correctly reflect other measures of quality control in the serology laboratory.

Other Measures of Quality Control. There are a number of other measures which are usually included as quality control steps in most serologic tests. Many of these measures are disregarded by the serologist in the interest of saving time and trouble, but invariably the quality of results suffers. Some of these important, yet frequently disregarded, measures are as follows:

a. Each new lot of antigen received by a laboratory should be checked for proper reactivity with a contro serum of known titer. The best method to use for evaluating a new lot of antigen is to set up duplicate routine runs using the old antigen in one run and the new antigen in the other. In this way, the new antigen can be well-characterized by comparison with the old antigen using a variety of serum specimens.

b. Proper pipetting techniques must be used. Best results are obtained by using point to point delivery with the pipette rather than draining liquid from the tip. Sera should not be mixed so vigorously that foaming occurs since this may denature some of the antibodies.

c. Serum should be removed from the clot as soon as possible and put in a clean, sterile tube. If not tested immediately, it should be refrigerated or frozen. In many cases, hemolyzed serum should not be used because of the possibility of false positive or aberrant results.

d. When there is a possibility of a prozone reaction, single dilution screening is not adequate. Several serum dilutions should be tested, with the least concentrated serum dilution beyond the level of the majority of prozone reactions.

e. When visual observations are made in determining results of serologic tests, optimal lighting conditions should be established for reading each type of test, and these conditions should be maintained from one time to the next to standardize results.

Thus far, we have discussed quality control in terms of the credibility of results obtained within the framework of individual laboratory tests. It is recognized that even when individual tests are thoroughly controlled, certain biases may occur during the routine run that can best be detected by a system of "internal control." These consist of a number of specimens of graded reactivity which are coded and randomly placed in the daily run so that special attention is not given to them. As in other controls, these specimens have known reactivity; when they are decoded at the end of the day, their results must fall within the acceptable range previously set for them.

The system of internal controls is quite similar to a proficiency testing program in which coded specimens of known reactivity are provided by an outside agency. Internal controls offer a means of comparing results of tests performed on different occasions within the same laboratory, whereas a proficiency testing program allows comparison of results obtained by several different laboratories. The former system is a further indicator of intra-laboratory precision of test results, and the latter system is an indicator of inter-laboratory comparability of test results. Both can be considered integral parts of a good quality control program.

Exercises (448):

Indicate whether each of the following statements is true (T) or false (F), and correct those that are false.

T F 1. The best method to use for evaluating a new lot of antigens is to set up duplicate routine runs using the old antigen is one run and the new in another.

T F 2. Best results are obtained by drawing the liquid from the tip of pipettes in serological techniques.

T F 3. Hemolyzed serum should be used with no possible effect on results.

T F 4. Vigorous mixing so that foam occurs enhances antigen-antibody reaction.

T F 5. When there is a possibility of a prozone reaction, a single dilution screening is adequate.

T F 6. When using "internal controls" in serology, results *must* fall within the acceptable range previously set for them.

T F 7. Internal controls offer a means of comparing results obtained by several different laboratories.

T F 8. A proficiency testing program is an indicator of inter-laboratory comparability of test results.

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Glossary

Absorption—The penetration or taking in of substances into the bulk of a solid or liquid. In blood banking and serology: the taking up of antibodies in a suspension onto the surfaces of particulate antigens.

ACD—Acid-Citrate-Dextrose Solution—An Anticoagulant, nutrient, and preservative used in the collection of blood for transfusion.

Achlorhydria—Absence of hydrochloric acid from the gastric sections.

Acquired Immunity—Immunity resulting from development of active or passive immunity.

Active Immunity—Immunity that results from formation within the body of substances which render a person immune.

Acute Phase—The early stage of an infectious disease in which the symptoms, including elevation of temperature, are manifest. With most infectious diseases this phase includes the first 3 to 5 days after initial onset of illness.

Adsortion—The property of a liquid or solid to retain or concentrate at its surface one or more components (atoms, molecules, or ions) from another solid or liquid. Blood banking and serology: the taking up of antibodies onto the surface of antigen particles.

Agglutination—The collection into clumps of cells or particles distributed in a fluid.

Agglutination Titer—The highest dilution of a serum that causes clumping of red blood cells.

Agglutinins—Antibodies that cause agglutination or clumping of cells in a suspension.

Agglutinogen—A red blood cell antigen.

AHG—Anti-human globulin (Coombs serum).

Albumin-Reactive Antibodies—Antibodies that fail to give a visible reaction with the corresponding red cells in saline but agglutinate the same cells suspended in bovine albumin.

Allele—One of two or more genes for a given trait at the same locus on homologous chromosomes.

Amboceptor—An immune substance that has an affinity for antigens and complement.

Anamnestic Reaction—An immunological reaction in which antibodies that have previously existed and have disappeared from the blood are rapidly redeveloped on the injection of the original antigen or a different nonspecific antigen.

Anemia—A condition in which the blood is deficient in quantity or quality of erythrocytes.

Anisocytosis—Variation in size of the erythrocytes.

Antibody—A specific substance which is produced in the body in response to the presence of an antigen and which reacts with the antigen in some certain way.

Antibody Combining Site—That particular area or arrangement of atoms on an immune body that determines its specificity.

Antigen—Any substance foreign to the body's antibody-forming tissues which stimulates these tissues to form antibodies.

Antigenic Determinant—Combining site on an antigen.

Antiserum—A serum (liquid portion of blood) containing antibodies.

Antistreptolysin O Titer—The level in the blood or tissues of specific antibodies against the streptolysin O hemolytic factor which is produced by certain streptococci.

Antitoxin—An antibody capable of neutralizing a specific toxin. It is produced in response to the presence of a toxin.

Au^a—Designation for the Auberger antigen.

Autoagglutination—Nonspecific clumping of an individual's cells by factors in his own serum or plasma. It is most common at low temperatures (5° C).

Autohemagglutinin (Autoagglutinin)—An antibody capable of agglutinating red blood cells in the same individual.

Autoimmune Disease—A disease characterized by the subject's body producing a substance foreign to its antibody-forming tissue, which is stimulated to produce antibodies against the substance.

Azurophilic Granule—Rounded, discrete, reddish-purple granule, smaller than the granules of neutrophiles; 0-10 are common in lymphocytes, and they are very numerous and smaller in the cytoplasm of monocytes.

Beef Erythrocyte Antigen—Substance contained in beef erythrocytes that is capable of absorbing the heterophile antibodies produced as a result of infectious mononucleosis and serum sickness.

Bilirubinemia—The level of the bile pigment bilirubin in blood.

Blocking Antibody—An antibody that coats red cells without promoting agglutination. This coating interferes with the agglutination of the erythrocytes suspended in saline by other antibodies specific for the red cell antigens.

Blood Grouping—Classification of blood specimens into groups (or types) on the basis of the red blood cell antigens.

Blood Group System—A system of related blood group antigens; e.g., the ABO system, the MNS system, the Rh-Hr system, etc.

Bombay Blood Group—The O$_h$ blood group that lacks H-substance.

Buffy Coat—The layer of white blood cells and platelets found overlying the red cells following packing by centrifugation.

Cardiolipin Antigen—Substance composed of extract from fresh beef hearts combined with lecithin and cholesterol. This antigen is used in flocculation and precipitation tests for syphilis.

Cardiolipin Microflocculation—A flocculation test for syphilis involving the reaction of syphilitic reagin with the cardiolipin antigen. The visible reaction appears as the aggregation of antigen particles to form floccules of varying size.

Chancre—A hard, syphilitic, primary ulcer. Usually the first sign of syphilis.

Chromatin—The deeply staining photoplasm of a cell's nucleus.

Chromosome—One of the dark staining, rod-shaped bodies appearing within the nucleus of the cell. It contains the genes.

Closed System—A term used to describe a blood pack or some special type of unit that is sealed under sterile conditions when it is filled and is not subsequently entered or opened in any way.

Cold Agglutinins—Agglutinins which react optimally at low temperatures.

Comatose—In a coma.

Compatibility Test—A test carried out between serum and erythrocytes to insure that they are not antagonistic. Usually this term refers to the major crossmatch between a patient's serum and donor's red cells.

Complement—A complex protein, present in fresh normal blood serum, which participates in various immunologic reactions. It is bound by antigen-antibody aggregates.

Complement Fixation—When antigen unites with its specific antibody, complement, if present, is taken into the combination and becomes inactive or fixed. Its presence or absence as free active complement can be shown by adding sensitized blood cells or blood cells and hemolytic amboceptor to the mixture. If free complement is present, hemolysis will occur; if not, no hemolysis will be observed.

Congenital—Born with a person; existing at or before birth.

Conjugate—In immunofluorescent microscopy, the union of antigen, antibody, or complement with the labeling substance (fluorescein).

Control—A controlled system used to test the accuracy of observations. In serological testing, controls are generally set up to check conditions and/or reagents.

Convalescent Phase—That stage of an infectious disease which immediately follows the cessation of clinical symptoms. In most infectious diseases this period is generally considered to be 10 to 14 days after the onset of illness.

CPD: Citrate-Phosphate-Dextrose Solution—A blood preservative, anticoagulant and nutrient used in the collection of blood for transfusion in which the 2, 3 - DPG concentrations are better maintained throughout a four week period than ACD.

C-Reactive Protein—A protein, not normally present in human blood which appears in a wide variety of inflammatory conditions. It is characterized by its ability to react visibly with the C-polysaccharide somatic substance of the pneumococci.

Crenation—The scalloped or notched appearance of the periphery of erythrocytes found when the cells are suspended in a hypertonic solution. Also found on smears and is caused by dirty glassware, slow drying, and poor smearing technique.

Cytolysis—Dissolution of cells by specific antiserum and complement.

Deoxyribonucleic Acid (DNA)—A substance containing a phosphoric ester found in the nuclei of all cells.

Determinant—That portion of an antigen molecule which determines the antigen's specificity but alone is not capable of eliciting an immune response.

Dextran—A polymer of dextrose $(C_6H_{12}O_6)_n$ used as a plasma volume expander. It is also sometimes employed as a cell-suspending medium for the detection of antibodies.

Differential Heterophil Test—A test designed to differentiate between the three types of heterophil antibodies.

Direct Antiglobulin Test (Direct Coombs Test)—A test for the detection of coated red blood cells (as a result of circulating antibodies) by the use of antihuman globulin.

Dominance—The expressed influence of one gene over another when two or more genes are present. For example, the gene for brown eyes shows a dominance over the gene for blue eyes. Blood group genes do not show dominance.

Dosage—Relative expression of gene action. For example, a stronger antibody reaction may be detected from homozygous (MM) individuals than from heterozygous ones (MN).

D^u—A variant of the Rho (D) antigen.

Dyscrasia—Abnormality.

Eluate—The antibody containing solution prepared by the elution of red cells.

Elution—The separation of adsorbed antibodies from the surface of red cells by the use of heat, and their placement in solution in the surrounding fluid medium.

Endemic Syphilis—A form of syphilis contracted through direct and indirect contact and limited to certain geographical areas.

Erythroblastosis Fetalis—See Hemolytic Disease of the Newborn.

Erythrocytosis—An increase in the total number of erythrocytes.

Erythropenia—A decrease in the total number of red blood cells in the blood.

Erythropoiesis—The production of erythrocytes.

Etiology—The theory of the causation of a disease.

Exchange Transfusion (Exsanguination Transfusions)—A method in which an infant's blood is withdrawn and simultaneously replaced by compatible blood. It is a means of treating hemolytic disease of the newborn.

Febrile Agglutinins—Agglutinating antibodies produced by the body in response to various fever-producing organisms. Examples are: antibodies directed against the causative agents of typhoid fever, paratyphoid, tularemia, undulant fever, typhus, etc.

Ficin—A proteolytic enzyme obtained from figs.

Flocculation—A phenomenon in which particles dispersed in a medium combine into discrete, usually visible aggregates.

Fluorescein—A red crystalline powder used to tag and identify antibodies in immunofluorescent microscopy.

Fluorescence—Luminescence of a substance when acted upon by short wave radiation. The substance absorbs light of certain wavelengths and emits light of a longer wave-length. The emission terminates simultaneously with the cessation of the incident exciting radiation.

Forssman Antibody—One of the heterophile antibodies which is naturally present in the blood of man in low titer.

Fy^a and Fy^b—Two antigens of the Duffy system.

Gamma Globulin—The fraction of serum globulin which contains most of the antibodies.

Gamete—Male or female reproductive cell.

GE—Designation for the Gerbich antigen.

Gene—The basic unit of inheritance, which carries one genetically transmissible character and which is arranged on a chromosome.

Genetics—The science of inheritance.

Genotype—The fundamental hereditary or genetic structure of an individual.

Group-Specific Substances—Carbohydrate substances found in the body fluids and tissues of persons whose erythrocytes contain the "A" and "B" antigens which inhibit anti-A and anti-B antibodies.

Guinea Pig Kidney Antigen—A substance used in the differential heterophile test. It absorbs the Forssman and serum sickness antibodies. The infectious mononucleosis antibody is absorbed to a much lesser degree or not at all.

Half-Life—The time required for a radioactive substance to lose half its energy.

Hapten (E)—That portion of antigenic molecule or antigen complex that determines its immunological specificity. It usually does not stimulate antibody formation by itself, but reacts specifically *in vivo* and *in vitro* with the antibody once it is formed.

Hemagglutination—The agglutination (clumping together) of red blood cells.

Hemagglutination-Inhibition—The prevention of the agglutination of red blood cells.

Hemagglutinin—Any immune substance which causes the agglutination of red blood cells.

Hematoma—A subcutaneous swelling containing effused blood. It can be caused by a traumatic venipuncture.

Hematopoiesis—The formation of red blood cells.

Hemolysin—An antibody capable of causing the lysis or dissolution of blood cells with the consequent release of hemoglobin.

Hemolysis—The lysis (dissolution) of red blood cells.

Hemolytic Anemia—That type of anemia characterized by excessive intravascular destruction of red blood cells.

Hemolytic Disease of the Newborn (HDN)—A disease manifested in infants as the result of *in utero* incompatibility between antigens of the fetus' red blood cells and maternal antibodies.

Hemostasis—The checking of the flow of blood, especially from a blood vessel.

Heparin—A substance that prevents coagulation of blood by stopping the conversion of prothrombin to thrombin and by neutralizing thrombin.

Heterophile Antibodies—A group of antibodies having an affinity for antigens found in a wide range of organisms. All heterophile antibodies have the common property of agglutinating sheep erythrocytes.

Heterozygous—A genotype composed of two different genes.

Homozygous—A genotype consisting of two identical genes.

H-Substance—A substance that is a precursor of the O gene.

Human Immune (Anti-D) Globulin—An immune serum globulin solution that is injected into a mother just after delivery to prevent Rh isoimmunization and subsequent hemolytic disease of the newborn.

Hypertonic Solution—A solution with an ionic concentration greater than the system with which it is compared.

Hypochromasia—A lack of hemoglobin in red blood cells.

Hypotonic Solution—A solution with an ionic concentration less than the system with which it is compared.

IgG— A synonym for 7-S or γG antibodies.

IgM—Another name for 19-S or γM antibodies.

Immune Bodies—Substances in those afflicted with antigenic disease agents formed by the tissues and capable of destroying or lessening the effect of the disease-producing agent. Also called antibodies.

Immune Response—The response of a body's antibody forming tissue to foreign antigens that results in the formation of antibodies.

Immunity—The state of being resistant to injury, particularly to foreign antigens, and is due to presence in the blood of specific antibodies, such as agglutinins, precipitins, opsonins, antitoxins, etc.

Immunoflurorescence—The technique of tagging antibodies with fluorescent dyes that aid in observing the reaction of these antibodies with specific antigens.

Immunogenic Agent—Any substance capable of inducing immunity or an immune response.

Immunoglobulin—That fraction of serum globulin containing antibodies which has the power to confer immunity.

Immunohematology—The study of the immune response with specific reference to blood.

Immunocompetent Cell—Any cell capable of forming immunoprotein substances (antibodies).

Immunology—The study of resistance to disease.

Immunoprotein—Any protein immune body (antibody) or substance that confers immunity.

Inactivation—The process by which the activity of serum complement is nullified. The usual means is to heat the serum to 56° C for 30 minutes.

Incompatibility—Agglutination or lysis of cells in the *in vitro* crossmatch procedure before the transfusion of blood.

Incomplete Antibody—An antibody that gives no visible reaction with red cells processing the corresponding specific antigen when these red cells are suspended in saline. The term is somewhat of a misnomer since the antibody itself is complete, but the conditions for its reaction with an antigen are unfavorable.

Infectious Mononucleosis—An acute infectious disease characterized by a sudden onset and acute course, with fever and inflammatory swelling of the lymph nodes, especially those of the cervical region. There is a moderate leukocytosis due almost entirely to abnormal mononuclear cells.

Intragroup Incompatibility—Incompatibility between individuals of the same ABO blood group: the cause of intragroup hemolytic reactions.

In Utero—In the uterus; during fetal life.

In Vitro—Outside of a living thing; i.e., observable in a test tube.

In Vivo—Within the living body.

Isoagglutinin—An antibody capable of only agglutinating cells of some individuals of the same species.

Isoantigens—Antigens found in some members of a species but not in other members.

Isoimmunization (Iso-Sensitization)—An antibody response to antigens from members of the same species.

Isotonic Solution—A solution with the same concentration as the system with which it is compared.

JK^a and JK^b—Antigens of the Kidd system.

K and k—Antigens of the Kell system.

Laked—Hemolyzed.

Le^a and Le^b —Designation for the Lewis-a and Lewis-b antigens, respectively.

Leukemia—A disease of the blood-fotming organs characterized by a marked increase in the number of leukocytes in the blood.

Leukemoid Reaction—A temporary increase in the number of immature leukocytes in the blood associated with a marked increase in the total leukocyte count.

Leukocytosis—An increase in leukocytes in the blood.

Leukopenia—A reduction in the number of leukocytes in the blood.

Linkage—Tendency for two or more genes to be inherited together.

Lipoproteins—A conjugated protein composed of a simple protein and a lipid.

Locus—The positin of a gene on a chromosome.

LU and Lu —Antigens of the Lutheran system.

Luminescence—Light emission that cannot be attributed to the temperature of the emitting body. It is sometimes characterized as fluorescent and phosphorescent. Phosphorescence is delayed light emission (afterglow), while fluorescence is emission during the period of excitation.

Lymphocytosis—A relative or absolute increase in the number of lymphocytes in the blood.

Lyophilization—The creation of a stable preparation of a biological material by rapid freezing and dehydration of the frozen product under high vacuum.

Lysin—An antibody that has the ability to cause dissolution or lysis of cells.

Lysozyme—A substance present in tears and other body fluids and tissues which destroy bacteria.

Macroglobulin—Any serum globulin with an unusually high molecular weight.

Major Crossmatch—The *in vitro* test between the donor's cells and the patient's serum.

Mendelian Laws—The basic laws of genetic inheritance proposed by Gregor Mendel.

Minor Crossmatch—The *in vitro* test between the patient's cells and the donor's serum.

Natural (Innate) Immunity—Most often, a permanent immunity that results from inherited factors.

Naturally Occurring Antibodies—Those antibodies that occur in a person shortly after birth and throughout adult life but are not produced by injections or pregnancy. They are due to "natural" environmental factors, probably proteins and polysaccharides, taken into the body via the digestive or respiratory systems.

Neutralization—An antigen-antibody reaction in which the reactive effect of a particular antigen is nullified by a specific antibody.

NIH—National Institute of Health.

Nonsusceptibility—Complete immunity to specific antigenic substances and is due to natural inherent factors.

Open System—A system, such as a blood pack unit, that has been opened or in some way exposed to the atmosphere. The use of transfer packs to withdraw serum from a blood unit renders the unit an open system.

Opsonin—A substance in blood serum which acts upon macroorganisms and other cells making them more attractive to phagocytes.

Panagglutinable Cells—Red cells that are agglutinated by sera from all adult human beings tested, regardless of the ABO groups.

Panagglutinating Serum—A serum causing the agglutination of all erythrocytes tested, irrespective of their blood group.

Panel of Cells—A series of separate erythrocyte suspensions of known antigenic content used to identify antibodies.

Papain—A proteolytic enzyme obtained from the tropical fruit paw-paw *(Carica papaya).*

Parachromatin—Any of several nonstaining or weakly staining nuclear elements.

Passive Immunity—Immunity produced by injection, absorption, or transfer of performed antibodies into the subject to be protected.

Pfeiffer Reaction—The lytic destruction of bacteria in the presence of immune serum and complement.

Phagocytin—A bactericidal substance, probably a globulin that can be isolated from polymorphonuclear leukocytes.

Phlebotomy—Withdrawal of blood from a vein.

Phenotype—The visible or physically observable inherited characteristics of an individual; for example, eye color, blood group.

Plasmapheresis—Return of packed red cells to the donor.

Poikilocytosis—An increased number of abnormally shaped erythrocytes.

Polychromasia—Diffuse basophilia of the erythrocytes.

Polychromatophilia—The presence on a stained blood smear of immature, nonnucleated, bluish staining red blood cells.

Polycythemia—An increase in the total number of red blood cells.

Polypeptide—A complex carbohydrate of high molecular weight found especially as a component of the capsule of various microorganisms.

Polysaccharide—Complex carbohydrates of high molecular weight found especially as a component of the capsule of various microorganisms.

Pooled Cells—A suspension of cells derived from several different donors and then placed together. Pooled cells are used in screening tests for the detection of antibodies.

Postzone Reaction—A weak or irregular antigen-antibody reaction occuring when a great excess of antigen is exposed to a serum containing a relatively low titer of antibody.

Potency of Antisera—The relative ability of sera to react with the specific antigen. High titer indicates great potency.

Precipitation—An antigen-antibody reaction in which a soluble antigen is caused to settle out by the action of its specific antibody.

Precipitin—An antibody formed in the body in response to soluble antigens. Precipitins react with soluble antigens to form insoluble precipitates or soluble complexes.

Precursor—That which precedes. Erythropoiesis: those cells that give rise to immature erythrocytes.

Presumptive Heterophile Test—A test to determine the presence of increased amounts of heterophile antibodies.

Properdin—A natural blood chemical that destroys bacteria and neutralizes viruses.

Prozone Phenomenon—A negative or weak antigen-antibody reaction occurring when serum containing a disproportionately high titer of antibody is exposed to a relatively small quantity of antigen.

Purpura—Small spots on the skin caused by subcutaneous effusion of blood.

Pyknotic Erythrocyte—An immature erythrocyte showing a dense compact nucleus and reduced cytoplasm.

Pyrogenic Reaction—A transfusion response, usually mild, due to pyrogens (q.v.).

Pyrogens—Substances that cause a fever. In blood they are filterable components, probably of bacterial origin, that may be present in the anticoagulant solution.

Reagin—An antibodylike substance produced by the body in response to certain types of tissue invasion and destruction. It is found in varying small amounts normally, but is usually increased in syphilis, malaria, and certain other diseases.

Recessiveness—The opposite of dominance (q.v.).

Reticuloendothelial System—Cells of the body with endothelial and reticular qualities that show a common phagocytic behavior toward foreign particles. This group includes endothelial and reticular cells of the spleen, lymph nodes, liver, and bone marrow.

Rh Antibodies (Wiener)—Anti-Rh , Anti-rh′, Anti-rh″, Anti-Hr , Anti-hr′, and Anti-hr″ (or Fisher-Race) Anti-D, Anti-C, Anti-E, and anti-d (theoretical), anti-c, and anti-e.

Rh$_o$ Blocking Serum—An Rh$_o$ antiserum containing antibodies of sufficient high titer and avidity to give a distinct blocking reaction.

Rheumatic Fever—A disease, probably infectious, associated with the presence of hemolytic streptococci in the body. Beginning with an attack of sore throat or pharyngitis, there develop chilliness, rapid rise of temperature, prostration, and painful inflammation of the joints.

Ribonuclear Proteins—A nucleoprotein that yields a ribonucleic acid on hydrolysis.

Rouleaux Formation (Pseudoagglutination)—The formation by human erythrocytes of stacks which appear microscopically as piles of coins. This "stacking" is enhanced by increased fibrinogen and/or globulin levels in the blood.

Secretors—Individuals who possess the "A" and "B" substances in their tissues and secrete it to their saliva, urine, tears, semen, gastric juice, and milk. These secretions are found in about 85 percent of the population.

Serial Dilution—Progressively higher dilutions of a substance arranged in a definite sequence or series.

Serology—The branch of biology which concerns itself with antigens and antibodies and their relationships.

Serum Sickness Antibody—Specific antibody produced in response to a foreign serum, especially if an illness results from the introduction of the foreign serum.

Sex Chromosome—X-chromosomes of a female and Y-chromosomes of a male which carry the genes that determine sex or are sex-linked.

Sorption—The process of taking up and holding either by adsorption or absorption.

Specificity—The special affinity of antigens for their corresponding homologous antibodies.

Spherocyte—A red blood cell which is more spherical, smaller, darker, and more fragile that normal.

Streptolysin O—An oxygen labile, hemolytic factor produced by certain streptococci.

Stroma—The spongy colorless supporting framework of an erythrocyte.

Stromalyzer—Any substance which causes dissolution of a cell's stromatin.

Thrombocytopenia—A decrease in the total number of thrombocytes.

Thrombocytosis—An increase in the number of circulating thrombocytes.

Thrombopoiesis—The formation of thrombocytes.

Thrombosis—Formation of a thrombus or blood clot.

Titer—An expression of the highest dilution of a substance that contains sufficient antibody material to give a visible reaction.

Titration—Determining the quantity of antibody in a serum by means of a serial dilution.

Trypsin—A proteolytic enzyme used for the detection of antibodies.

Vasoconstrictor Substances—Substances that cause constriction of blood vessels.

Ve^a —Designation for the Vel antigen.

Venereal Syphilis—A form of syphilis contracted through direct sexual relations.

Warmed Autoantibody—A variety of autoantibodies reacting with equal intensity at body and refrigerator temperatures. Such antibodies do not occur normally, but appear to be produced only by certain individuals who have a remarkable capacity to produce antibodies in general. This may give rise to the disease, acquired hemolytic anemia.

Washed Cells—Cells freed of plasma or serum by repeated centrifugation through fresh volumes of normal saline.

Weil-Felix Reaction—The diagnostic agglutination of Proteus X bacteria by the blood sera of typhus cases due to the presence of an antigen in the bacteria common to that found in the causative rickettsial organisms.

Widal Test—A procedure designed to detect antibodies, if present, against the causative organisms of typhoid fever.

Xanthrochromia—A yellow discoloration.

Yt^a—Designation of the Cartwright antigen.

ANSWERS FOR EXERCISES

CHAPTER 1

Reference:

400 - 1. A body of knowledge concerned with the biological, chemical, and physical factors that contribute to the body's resistance to immunogenic agents.
400 - 2. No. A microorganism is an immunogenic agent *only* if capable of causing an immune response which results in the production of antibodies.
400 - 3. a. The state of hypersensitivity.
b. The rejection of allografts.
c. Grafts versus host reactions.
d. Other phenomena related to our intolerance of many things foreign when they occur within our bodies.
400 - 4. Pollens, foreign proteins, and venoms.
400 - 5. Polysaccharides, lipids, and other chemical entities.

401 - 1. The study of serum. This is accomplished through the use or detection of serum globulins known as antibodies.
401 - 2. a. To detect and measure antibodies in autoimmune disease.
b. To perform immunoassay of hormones and immunoglobulins.
c. To improve prognosis in organ or tissue transplants by determining the compatibility level between recipient and donor.
d. To detect certain tumor antigens.

402 - 1. Immunity dependent upon some special property of a particular animal species rather than to a specific antibody.
402 - 2. Human beings possess an innate or natural resistance to this disease whereas cows do not.

403 - 1. F. Features are physiologic and anatomic, not dependent upon antibodies.
403 - 2. T.
403 - 3. F. Will not.
403 - 4. T.

404 - 1. c.
404 - 2. j.
404 - 3. j.
404 - 4. j.
404 - 5. d.
404 - 6. d.
404 - 7. f.
404 - 8. h, b.
404 - 9. b.
404 - 10. e.
404 - 11. a.
404 - 12. g.
404 - 13. i.
404 - 14. i.

405 - 1. The type of immunity that is obtained or developed after birth.
405 - 2. Active immunity.
405 - 3. It is called active immunity because the individual actively produced antibodies himself at some stage in his life.
405 - 4. Naturally-acquired active immunity.
405 - 5. Artificially-acquired active immunity.
405 - 6. 1. Short-lived immunity: gonorrhea and influenza.
2. Life-long immunity: measles and chicken pox.
405 - 7. The rise in antibody titer.
405 - 8. His test will not usually differentiate between the two.

406 - 1. In active immunity, antibodies have been produced as a result of previous infection. In passive immunity, the antibodies are gained from an outside source such as an injection.
406 - 2. The antibodies may pass from the immune mother to the fetus across the placental barrier, or from its mother's milk which is rich in antibodies for a short time after birth.
406 - 3. The newborn is incapable of producing antibodies of his own for a few months after birth.
406 - 4. It is used for prophylaxis following exposure to such diseases as rubella and infectious hepatitis.
406 - 5. This is usually accomplished by injecting the recipient with antibodies (gamma globulin) which have been extracted from the blood of immune persons.
406 - 6. When passive immunity is gained through produced antibodies, the person receiving them does not undergo immune response as a result of receiving such immunity. Consequently, as soon as these transferred antibodies are removed from the blood, the passive immunity will no longer exist.

407 - 1. Any foreign substance that stimulates the body (or any animal's body) to produce antibodies.
407 - 2. The lens is not in intimate contact with the reticuloendothelial system which produces the antibodies, so the lens is, therefore, recognized as foreign.
407 - 3. It is associated with some malfunction of antibody production or recognition.
407 - 4. 10,000.
407 - 5. Proteins or protein complexes with carbohydrates or lipids.
407 - 6. The carrier and the determinant.
407 - 7. They provide specificity for the antigen, stimulate the body to produce specific antibodies against them, and are the reaction sites for the antibodies in antigen-antibody reactions.
407 - 8. Reactivity with antibodies or the utilization and observation of the antigen-antibody reaction.
407 - 9. They are rapidly localized in fixed macrophages (phagocytic histiocytes) of the liver, spleen, and bone marrow.
407 - 10. Antigens; lymphocytes.

408 - 1. F. Complex proteins.
408 - 2. T.
408 - 3. F. High.
408 - 4. F. Globulin.
408 - 5. T.

409 - 1. IgG, IgM, IgD, IgA, and IgE.
409 - 2. IgG, IgM, and IgA.
409 - 3. IgM.
409 - 4. IgG.
409 - 5. IgM.
409 - 6. IgG.
409 - 7. Light; heavy.
409 - 8. Ten light chains and ten heavy chains.
409 - 9. The variable region.
409 - 10. Alpha (α), beta (β), and gamma (γ).
409 - 11. Both antigens and antibodies are high molecular weight substances and protein in nature.
409 - 12. They are separated according to sedimentation constant, which is a code for differentiating them according to molecular weight.
409 - 13. Immunoelectrophoresis.

410 - 1. d.
410 - 2. d.
410 - 3. d.
410 - 4. a, e.
410 - 5. a, e.
410 - 6. c.
410 - 7. b.
410 - 8. c.
410 - 9. d.
410 - 10. b.

411 - 1. Lymphocytes and plasmocytes.
411 - 2. A cell that is capable of forming an antibody.
411 - 3. The indirect template theory.
411 - 4. The direct template theory.
411 - 5. An aggregate of cells, all of which are descended from a single parent cell.

412 - 1. F. Whole blood also.
412 - 2. T.
412 - 3. T.
412 - 4. F. Guinea pig.
412 - 5. T.
412 - 6. F. Complement becomes fixed in bacteriolytic and hemolytic reactions also.
412 - 7. F. Sensitized blood cells.
412 - 8. T.
412 - 9. F. Globulin components.
412 - 10. T.
412 - 11. F. Irreversible.
412 - 12. F. C9.

413 - 1. Antigen-antibody complementary fix.
413 - 2. Ionic bonds, hydrogen bonds, Van der Waals forces, and hydrophobic bonds.

413 - 3. Yes. Reactions may be reversed. In reactions in which binding forces are weak, reactions may be reversed or unbound.
413 - 4. It implies that the reaction is between components that possess particular properties that allow them to react. We have referred to these special properties as complementary fix.
413 - 5. Antibodies are produced which react with identical or nearly identical antigens from two or more unrelated organisms.
413 - 6. Cross reactions are useful in identifying certain antigens or antibodies.
413 - 7. a. Salmonella typing.
    b. The use of sheep and horse red blood cells with which antibodies produced by infectious mononucleosis will react.
    c. Use of certain strains of Proteus organism as antigens in serological testing for rickettsial diseases.

414 - 1. T.
414 - 2. F. Weak or negative reactions.
414 - 3. F. Before making dilution of the antibodies (serum) before adding the antigen.
414 - 4. F. Antigen.
414 - 5. T.

415 - 1. F. Several dilutions.
415 - 2. F. It is usually a cell suspension.
415 - 3. F. Defines titer.
525 - 4. F. Tube.
415 - 5. T.
415 - 6. T.

416 - 1. Humans, cows, sheep, chickens, guinea pigs, and horses.
416 - 2. Saline or albumin.
416 - 3. Use the formula for finding PCV and substitute known values into the formula.

Packed cell volume

$$= \frac{\text{total volume X \% solution desired}}{100}$$

$$= \frac{75 \times 3}{100} = \frac{225}{100} = 2.25 \text{ ml.}$$

q.s. 2.25 ml. PCV to 75 ml. with diluent.

416 - 4. The second formula applies here.

$$\text{\% cell suspension} = \frac{\text{PCV} \times 100}{\text{total volume}}$$

$$= \frac{4.4 \times 100}{100} = 4.4\%$$

416 - 5. Apply the last formula, the one that finds an unknown total volume, in this instance.

$$\text{Total volume} = \frac{\text{PCV} \times 100}{\text{\% solution desired}}$$

$$= \frac{0.36 \times 100}{3} = \frac{36}{3} = 12 \text{ ml. total volume}$$

416 - 6. a. Cutting short or altering cell washing techniques.
    b. Skimping.
    c. Use of old cells.
    d. The use of your finger to cover the tube opening when mixing.

417 - 1. Sequential reduction of concentration in mathematical progression.
417 - 2. Twofold.
417 - 3. One; nineteen; twenty.
417 - 4. Calibrated loop.
417 - 5. $\text{Dilution} = \frac{\text{total volume in tube}}{\text{volume of serum in tube}}$

417 - 6. $\text{Dilution} = \frac{\text{total volume}}{\text{volume of serum in tube}}$

$$= \frac{10}{.025} = 400 = 1{:}400$$

417 - 7. Before, after.

418 - 1. c.
418 - 2. c.
418 - 3. a, d.
418 - 4. a.
418 - 5. f.
418 - 6. e.
418 - 7. b.
418 - 8. b.

419 - 1. These tests are based on the fact that certain chemicals emit visible light when exposed to ultraviolet (UV) light.
419 - 2. Fluorescein isothiocyanate.
419 - 3. Direct fluorescent antibody technique.
419 - 4. Fluorescein-labeled antihuman globulin that has been produced in an animal, following the injection of human globulin.
419 - 5. The Fluorescent Treponemal Antibody Absorption (FTA-ABS) test for syphilis and the Antinuclear Antibody Test for lupus erythematosus.
419 - 6. It is less sensitive than the indirect method and would necessitate the tedious process of conjugation of sera of individual patients.

CHAPTER 2

420 - 1. An isophile antibody is one that reacts only with antigens of certain members of a species. A heterophile antibody is one that reacts with an antigen that is common to all members of a species.
420 - 2. Forssman antibodies.
420 - 3. Serum sickness antibodies.
420 - 4. Infectious mononucleosis antibodies.

421 - 1. F. Forssman antigens stimulate the production of sheep agglutinins.

421 - 2. F. It is absent in these animals.
421 - 3. F. It is present in these animals.
421 - 4. T.
421 - 5. F. Serum sickness produced in response to an injection containing horse serum.
421 - 6. T.
421 - 7. T.
421 - 8. T.
421 - 9. F. Inactivation keeps the test from showing a hemolytic reaction.

422 - 1. F. Some cases.
422 - 2. F. Virus.
422 - 3. F. Virus.
422 - 4. F. EB virus.
422 - 5. T.
422 - 6. T.
422 - 7. F. It is found to be sensitive, reproducible, and specific.
422 - 8. T.

423 - 1. IM antibodies were not removed by guinea pig kidney.
423 - 2. The IM antibodies were completely absorbed by beef erythrocytes.
423 - 3. They observed that infectious mononucleosis caused the production of antibodies in higher titer than those of Forssman or those caused by serum sickness.
423 - 4. To inactivate complement.
423 - 5. If complement is not removed, it lets sheep hemolysin react and causes hemolysis that might give misleading results.
423 - 6. A source of heterophile antigen and as an indicator of reaction.
423 - 7. The specimen is considered negative for IM antibodies.
423 - 8. A differential heterophile test is required.

424 - 1. Macerated guinea pig kidney and boiled beef erythrocytes.
424 - 2. Infectious mononucleosis.
424 - 3. Infectious mononucleosis and serum sickness.
424 - 4. It differs by no more than a three-tube drop as compared to the presumptive test.
424 - 5. Incomplete inactivation of complement in the specimen.

425 - 1. F. Beef erythrocytes and complement.
425 - 2. T.
425 - 3. T.
425 - 4. F. The untreated cells agglutinate and the papainized cells do not agglutinate.
425 - 5. T.
425 - 6. F. Acceptable.
425 - 7. T.
425 - 8. F. Liver function tests.

426 - 1. Acute and convalescent samples.
426 - 2. An incidental immune response to a previous immunizing agent.
426 - 3. The patient's history and know some characteristics of the causative organisms.
426 - 4. Several strains of *S. paratyphi A, S. paratyphi B,* and *S. choleraesuis.*
426 - 5. Plague, brucellosis, tularemia, typhus, Rocky Mountain spotted fever, and typhoid fever.

427 - 1. It is a general term for agglutination tests for all salmonellosis, including typhoid fever and paratyphoid fevers.
427 - 2. An electrolytic solution such as saline is used in the test.
427 - 3. Usually with heat or phenol.
427 - 4. The strain of organism.
427 - 5. Other salmonella species than *Salmonella typhi,* such as Group B.
427 - 6. The O antigen.
427 - 7. The O antigen.
427 - 8. The flagellar antigen-antiboey reaction is soft and fluffy. It is easily broken up by vigorous shaking.
427 - 9. The titer of O agglutinins rises earlier in the disease and then drops faster than H agglutinins.
427 - 10. Group B, C_1, C_2, and D.
427 - 11. Group B.

428 - 1. The reaction in which some of the rickettsial diseases stimulate the production of bacterial agglutinins against certain nonmotile *Proteus* OX strains.
428 - 2. No. The antigen used in the test is a *Proteus* antigen. The test detects antibodies produced in response to rickettsial diseases.
428 - 3. Proteus, OX-19, OX-2, and OX-K.
428 - 4. The antigen is a somatic antigen of this nonmotile strain.
428 - 5. A significant rise in titer from several specimens taken.
428 - 6. *Brucella abortus* and *Francisella (Pasteurella) tularensis.*
428 - 7. Serologically.
428 - 8. a. The disease prevalence of the geographic location.
    b. Patient's history.
    c. Symptoms.
    d. The availability of antigens.
428 - 9. *Salmonella* O Group A and *Proteus* OK-K.

429 - 1. F. Large amount of time involved in diluting and pipetting and the long incubation time.
429 - 2. T.
429 - 3. T.
429 - 4. T.
429 - 5. F. The tube test should be performed.
429 - 6. T.

430 - 1. T.
430 - 2. F. It's different from lobar pneumonia.

430 - 3. F. *Mycoplasma pneumoniae.*
430 - 4. T.
430 - 5. F. It is a slow process and most cases of PAP will be diagnosed serologically.

431 - 1. c.
431 - 2. b.
431 - 3. b.
431 - 4. e.
431 - 5. e.
431 - 6. d.
431 - 7. a.
431 - 8. d.
431 - 9. a.
431 - 10. a.

432 - 1. F. 37° C.
432 - 2. T.
432 - 3. F. Separate immediately from the clot.
432 - 4. F. Group O.
432 - 5. T.
432 - 6. F. Read immediately, since warming may cause agglutination to disappear.
432 - 7. T.
432 - 8. T.

CHAPTER 3

433 - 1. F. A chronic inflammatory disease affecting joints and synovial membranes.
433 - 2. F. Early symptoms of joint pain and muscular stiffness are not unique to rhematoid arthritis.
433 - 3. T.
433 - 4. F. Are mainly against IgG.
433 - 5. T.
433 - 6. F. Does not rule out the possibility—20 percent of actual cases have negative results.
433 - 7. T.
433 - 8. F. Detecting increases in titer is especially significant.

434 - 1. c.
434 - 2. a.
434 - 3. a.
434 - 4. d.
434 - 5. b.
434 - 6. e.

434 - 7. e.
434 - 8. e.
434 - 9. c.

435 - 1. l.
435 - 2. l.
435 - 3. m.
435 - 4. e.
435 - 5. e.
435 - 6. f.
435 - 7. d.
435 - 8. c, d.
435 - 9. g, i.
435 - 10. h, j, k, m.
435 - 11. a.
435 - 12. b.

436 - 1. F. Second-stage reaction.
436 - 2. T.
436 - 3. F. Immunodiffusion in agar.
436 - 4. T.
436 - 5. F. The precipitate forms at the antigen-antibody interface or throughout the mixture.
436 - 6. F. Insensitive; it detects only large amounts of antibodies.
436 - 7. T.

437 - 1. These tests are based on the migration of the antigen and/or antibody through the agar until optimum proportions of each are reacted and a precipitate appears.
437 - 2. Electrophoresis.
437 - 3. Double diffusion.
437 - 4. The antigen and antisera are placed in separate wells cut into the agar and each diffuses toward the other.
437 - 5. Coccidiodomycosis.
437 - 6. The antibody is incorporated into the agar.
437 - 7. The antigen is placed in a well and diffuses radially into the agar until a precipitin line is formed.
437 - 8. A ring of precipitate forms whose diameter is proportional to the antigen concentration.
437 - 9. Immunoglobulins and complement.
437 - 10. Counterelectrophoresis and counter-current electrophoresis.
437 - 11. IEOP is a combination of simultaneous diffusion and electrophoresis followed by precipitation of reactants.
437 - 12. Antigen and antibody are placed in separate wells in the agar and electrical current is applied.
437 - 13. The pH ionic strength of the agar and the nature of the antigen and antibody.
437 - 14. Increased speed and the ability to detect very small quantities of antigen.
437 - 15. The detection of hepatitis associated antigen (HB$_s$Ag).

438 - 1. T.
438 - 2. F. the standardized streptolysin O solution will be neutralized and the blood cells will *not* be hemolyzed.
438 - 3. T.
438 - 4. T.
438 - 5. F. 100 Todd units.
438 - 6. T.
438 - 7. T.
438 - 8. T.
438 - 9. F. Wash cells thoroughly to remove residual antistreptolysin O.
438 - 10. T.

439 - 1. F. Extracellular products.
439 - 2. F. Group A.
439 - 3. F. Streptococcus MG antibodies are associated with primary atypical pneumonia.
439 - 4. T.
439 - 5. F. The ASO test.
439 - 6. T.
439 - 7. T.
439 - 8. F. 1:166 dilution or 166 Todd units.

CHAPTER 4

440 - 1. F. Three weeks.
440 - 2. F. Darkfield examination.
440 - 3. F. It does not.
440 - 4. F. Incorrect decision; this can be dangerous because inadequate treatment in the primary stage will mask symptoms and lead to the more serious secondary stage.
440 - 5. T.
440 - 6. F. CSF must be nonreactive.
440 - 7. F. The latent stage.
440 - 8. F. The latent syphilis.
440 - 9. T.
440 - 10. T.
440 - 11. T.
440 - 12. F. It is possible.
440 - 13. F. Survival is unlikely under such storage conditions.
440 - 14. T.
440 - 15. F. Environmental Health Services of the hospital and indirectly involves the laboratory.
440 - 16. F. None or very little.

441 - 1. Endemic syphilis is spread by direct and indirect contact.
441 - 2. It is rare in endemic syphilis. Fatalities are also rare.
441 - 3. Yaws, pinta, and rat-bite fever.
441 - 4. *Treponema pertenue*.
441 - 5. It does not involve the viscera or central nervous system; congenital transmission is rare; it's predominantly a childhood disease.
441 - 6. Pinta.
441 - 7. *Streptobacillus moniliformis* and *Spirocheta morsus muris*.

442 - 1. Nontreponemal (reagin) tests and treponemal tests.
442 - 2. Cardiolipin, an extract of beef heart, mixed with lecithin and cholesterol.
442 - 3. Carbon particles (charcoal).
442 - 4. Reagins; reaginic antibodies.
442 - 5. Malaria, leprosy, hepatitis, and certain immunizations.
442 - 6. Biological false positives; BFP.
442 - 7. Reactive, Weakly Reactive, or Nonreactive.
442 - 8. Should not be.
442 - 9. Helpful in following the effectiveness of therapy.
442 - 10. Biological false positives are essentially eliminated.
442 - 11. a. Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test.
    b. Treponema Pallidum Immobilization (TPI) Test.
    c. Reiter Protein.Complement Fixation (RPCF) Test.

443 - 1. The presence of reaginic antibodies in the serum.
443 - 2. Increasing the antigen's effective reacting surface.
443 - 3. 10; 56° C.
443 - 4. Reactive.
443 - 5. a. A prior technical problem.
    b. A temporary biological false positive in the patient.
443 - 6. 6 to 8 weeks.
443 - 7. It decreases until it reaches a fairly stable level during the late course of the disease.
443 - 8. Confusing precipitates.
443 - 9. To insure an even suspension of antigen particles.

444 - 1. 0.05; 18.
444 - 2. 1/60; carbon-containing RPR antigen.
444 - 3. 8.
444 - 4. Humidifying cover.
444 - 5. Reactive; titer.
444 - 6. Decrease; increase.
444 - 7. Blow.
444 - 8. Alter.
444 - 9. Possible.
444 - 10. Plasma.

445 - 1. F. 10 percent.
445 - 2. F. 0.05 ml of unheated spinal fluid.
445 - 3. F. Microscopically.
445 - 4. T.

445 - 5. F. 10 percent.
445 - 6. *F. Specimen unsatisfactory.*
445 - 7. T.
445 - 8. T.
445 - 9. F. Nonreactive.
445 - 10. T.
445 - 11. T.
445 - 12. T.

446 - 1. c.
446 - 2. e.
446 - 3. e.
446 - 4. d.
446 - 5. d.
446 - 6. b.
446 - 7. b.
446 - 8. b.
446 - 9. b.
446 - 10. f.
446 - 11. f.

447 - 1. F. They can be affected.
447 - 2. T.
447 - 3. *T.*
447 - 4. F. High level noise tends to distract and may result in errors.
447 - 5. F. Daily.
447 - 6. F. Replaced.
447 - 7. T.
447 - 8. F. You should write your own initials on the label.
447 - 9. T.
447 - 10. F. Outdated reagents should not be used and must be replaced.

448 - 1. T.
448 - 2. F. Using point to point delivery.
448 - 3. F. False positive results or aberrant results are possible.
448 - 4. F. This may denature some of the antibodies.
448 - 5. F. Several dilutions should be tested.
448 - 6. T.
448 - 7. F. Test performed on different occasions within the same laboratory.
448 - 8. T.

AU GAFS, AL. (834468)1600

STOP-

1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.

2. USE NUMBER 2 PENCIL ONLY.

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EXTENSION COURSE INSTITUTE
VOLUME REVIEW EXERCISE
SEROLOGY

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that item numbers on answer sheet are sequential in each column.
3. Use a medium sharp #2 black lead pencil for marking answer sheet.
4. Write the correct answer in the margin at the left of the item. (When you review for the course examination, you can cover your answers with a strip of paper and then check your review answers against your original choices.) After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if a all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DONT'S:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than a #2 black lead pencil.

NOTE: NUMBERED LEARNING OBJECTIVE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Learning Objective Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Learning Objectives* indicated by these *Numbers.* The VRE results will be sent to you on a postcard which will list the *actual* VRE *items you missed.* Go to the VRE booklet and locate the *Learning Objective Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (400) What body of knowledge is concerned with the biological, chemical, and physical factors that contribute to the body's resistance to immunogenic agents?

   a. Sensitization.
   b. Antigenology.
   c. Immunology.
   d. Serology.

2. (400) The following reactions must be included under immune responses except

   a. grafts versus host reaction.
   b. intolerance to climatic changes in our environment.
   c. the state of hypersensitivity.
   d. the rejection of allografts.

3. (402) What type of immunity depends upon special properties of a particular animal species rather than a specific antibody?

   a. Native immunity.
   b. Natural resistance.
   c. Natural immunity.
   d. Passive immunity.

4. (404) A substance which functions as a body defense mechanism, has a high molecular weight, is effective against certain viruses, and has gram positive and gram negative bacteria is

   a. properdin.
   b. interferon.
   c. lysozyme.
   d. mucus.

5. (404) Which of the following body defense mechanisms specificity of an antibody, attacks many different organisms, and has been called a "natural antibody"?

   a. Lysozyme.
   b. Mucus.
   c. Interferon.
   d. Properdin.

6. (405) When antibodies are produced after an actual infection occurs, what type of immunity is acquired?

   a. Naturally-acquired active.
   b. Naturally-acquired passive.
   c. Natural resistance.
   d. Native resistance.

7. (406) What term best describes the duration of passive immunity?

   a. Forever.
   b. Several years.
   c. 1 year.
   d. A short time.

8. (406) If possible, how does an infant acquire antibodies from the mother shortly after birth?

   a. The infant cannot acquire antibodies.
   b. The infant has no need for antibodies.
   c. From the mother's milk.
   d. Across the placental barrier.

9. (407) All of the following statements concerning antigens are true except that antigens

   a. stimulate the formation of specific antibodies.
   b. are usually formed by the globulin fraction of serum protein.
   c. usually have molecular weights of 10,000 or more.
   d. are protein complexes with carbohydrates or lipids.

10. (407) The determinant, as one of the two parts of an antigen, serves all of the following purposes except to

a. provide complex polysaccharides for reaction with antibodies.
b. provide reaction sites for antibodies in antigen-antibody reactions.
c. stimulate the body to produce specific antibody against them.
d. provide specificity for the antigen.

11. (408) More specifically, most antibody activity is found in what fraction of serum globulin?

a. Fibrinogen.
b. Albumin.
c. Gamma globulin.
d. Beta globulin.

12. (409) What are the primary types of antibodies against bacteria endotoxins?

a. IgM.
b. IgA.
c. IgD.
c. IgG.

13. (409) The Greek characters α, β, and γ are used to identify fractions of what substance?

a. Serum.
b. Blood.
c. Globulin.
d. Albumin.

14. (409) Structurally, each immunoglobulin molecule consists of light and heavy

a. polysaccharides.
b. amino acids.
c. clones of mesenchymal cells.
d. nucleic acids.

15. (410) Which of the following is a differentiating characteristic of the immunoglobulin fraction IgG?

a. IgG occurs in large amounts of tears.
b. IgG is produced after immunization but diminishes as a strong IgM response takes over.
c. IgG molecules are small enough to cross the human placenta.
d. IgG occurs in internal secretions and may provide protection where other types do not.

16. (410) Identify the immunoglobulin which is the first antibody to be detected after a primary infection.

a. IgM.
b. IgA.
c. IgG.
d. IgE.

17. (411) In which template theory of antibody production is the genetic memory of a cell affected by the presence of antigen within the cell?

a. The direct template theory.
b. The indirect template theory.
c. The natural template theory.
d. The immunogenic theory.

18. (412) How can complement best be described?

a. An immunogenic agent.
b. An immunoprotein.
c. A lytic substance.
d. A neutralizing antibody.

19. (412) In the Pfeiffer reaction, complement was found to be

a. oxygen stable.
b. oxygen labile.
c. heat stable.
d. heat labile.

20. (412) Complement-fixation is the reaction of

a. antigen and complement only.
b. antibody and complement only.
c. antigen, complement, and antibody.
d. bacteria and complement.

21. (412) Complement increases the susceptibility of bacteria to phagocytosis through what activity?

a. Lysis.
b. Hemolysis.
c. Opsonization.
d. Neutralization.

22. (413) The "Lock and Key" concept has been used to describe what feature of antigens and antibodies?

a. Antigen-antibody equivalence zone.
b. Antigen-antibody complementary fit.
c. Antigen-antibody optimum zone.
d. Antibody-complement equivalence zone.

23. (413) Antigen-antibody complex is held together by all of the forces except which of the following?

a. Ionic bonds.
b. Hydrogen bonds.
c. Van der Waals forces.
d. Immunogenic forces.

24. (413) When antibodies are produced which react with identical antigens from two unrelated organisms, what reaction is produced?

a. Complementary.
b. Cross-reactivity.
c. Complementary fit.
d. In vivo.

25. (414) Which of the following serological reactions are likely to be seen early in a disease before an optimal antibody level is achieved?

a. Equivalence zone.
b. Optimum zone.
c. Prozone.
d. Postzone.

26. (414) In a prozone, a weak or false negative result is due to which of the following conditions?

a. An excess of antigen.
b. Diluted antigen.
c. An excess of antibody.
d. Diluted antibody.

27. (416) What volume of packed cells is required to make 70 ml of a 2-percent cell suspension?

a. 0.7.
b. 14.0.
c. 1.4.
d. 0.14.

28. (417) The loop used in the microtitration technique must be cleaned in

a. alcohol.
b. hot, soapy water.
c. a flame.
d. a saline solution.

29. (417) You are calculating a dilution and have a tube in which 0.0014 ml of serum is diluted to a total volume of 1 ml. The dilution of the serum would be

a. 1:714.
b. 1:514.
c. 1:314.
d. 1:114.

30. (418) Complement-fixation tests are used to detect fixation of complement by which of the following components?

a. Globulins.
b. Antigens.
c. Immunogenic protein.
d. Antibodies.

31. (418) Which of the following types of serological reactions is best demonstrated in the antistreptolysin O test?

a. Agglutination.
b. Immunodiffusion.
c. Neutralization.
d. Complement fixation.

32. (419) In the indirect fluorescent antibody technique, prior to examination by the UV microscopy, what substance is added after the excess antibody has been washed off the known antigen on the slide?

a. Merthiolate labeled agar.
b. Fluorescein labeled agar.
c. Fluorescein labeled antihuman globulin.
d. Merthiolate labeled antihuman globulin.

33. (420) The three types of heterophile antibodies are

a. serum sickness, Forssman, and infectious mononucleosis.
b. antiglobulins, antitoxin, and infectious mononucleosis.
c. infectious mononucleosis, isophile, and Forssman.
d. Forssman, isophile, and serum sickness.

34. (420) Forssman antibodies develop mainly from exposure to which of the following?

a. Serum proteins.
b. Horse serum.
c. Isophile antigens.
d. Plants and bacteria.

35. (421) Serum sickness antibodies are produced in response to injections containing

a. strains of Salmonella.
b. strains of Shigella.
c. horse serum.
d. guinea pig serum.

36. (422) Studies have implicated which of the following as a possible cause of infectious mononucleosis?

a. Epstein-Barr virus.
b. Bacterial organism.
c. *Listeria monocytogenes*.
d. Malignant lymphoma.

37. (423) We call the Paul-Bunnell test a presumptive test because if the titer is 1:224 or higher, we assume which one of the following is present in the serum?

a. Infectious mononucleosis antibodies.
b. Antisheep cell hemolysins.
c. Serum sickness antibodies.
d. Forssman antibodies.

38. (423) During the second to fourth week of atypical infectious mononucleosis infection, the patient's titer is usually

a. lowest.
b. variable.
c. highest.
d. not detectable.

39. (423) A patient who has *not* received an injection of horse serum is considered to have IM if the presumptive titer is

a. 28.
b. 56.
c. 112.
d. 224.

40. (424) In the Davidsohm differential test, what other suspension besides macerated guinea pig kidney is used to absorb heterophile antibodies?

a. Boiled beef erythrocytes.
b. Boiled beef kidney.
c. Guinea pig erythrocytes.
d. Boiled horse erythrocytes.

41. (425) In the Ox hemolysin test, for infectious mononucleosis, what other substance besides beef erythrocytes is added to serially diluted serum?

a. Latex particles.
b. Guinea pig kidney.
c. Complement.
d. A proteolytic enzyme.

42. (425) When used to detect infectious mononucleosis antibodies, which are the most sensitive and have fewer false positives than sheep erythrocytes?

a. Guinea pig erythrocytes.
b. Horse erythrocytes.
c. Ox cells.
d. Rabbit erythrocytes.

43. (427) The Widal reaction is a general term for agglutination tests including which of the following diseases?

a. Brucellosis.
b. Tularemia.
c. Rocky Mountain spotted fever.
d. Salmonelloses.

44. (427) Care should be exercised when reading flagellar antigen-antibody reactions because the flagellar antigen-antibody reaction is

a. thermolabile.
b. unstable due to the size of the antigens used.
c. soft and fluffy and is easily broken up.
d. brittle and easily broken up.

45. (428) The _Proteus_ "O" antigens are

a. somatic antigens.
b. antityphus antigens.
c. rickettsial agglutinins.
d. nonspecific.

46. (428) The Weil-Felix test is considered diagnostic if

a. the titer is 1:80.
b. reaction is with a single strain of _Proteus_.
c. there is a rise in titer.
d. no performed antibodies are present.

47. (430) Primary atypical pneumonia is strongly associated with which of the following organisms?

a. _Streptococcus pneumoniae_.
b. _Mycoplasma pneumoniae_.
c. _Staphylococcus aureus_.
d. _Salmonella typhi_.

48. (431) In which of the following serologic tests for detecting antibodies in primary atypical pneumonia is the organism _M. pneumoniae_ added to the patient's serially diluted serum in a medium containing tetrazolium?

a. Immunofluorescent.
b. Growth inhibiting.
c. Indirect hemagglutination.
d. Streptococcus MG.

49. (431) In a test for primary atypical pneumonia, what type of antibody is rarely encountered which causes false positives against the antigen?

a. Cold agglutinins.
b. Streptococcus MG agglutinins.
c. Immunofluorescent antibodies.
d. Indirect hemagglutination antibodies.

50. (432) Since cold agglutinins can be absorbed from the serum by the patient's RBCs, until the serum can be separated, the blood must be maintained at what temperature?

a. 4° C.
b. 10° C.
c. 25° C.
d. 37° C.

51. (433) About eighty percent of classical cases of rheumatoid arthritis have which of the following types of globulin antibodies against what specific immunoglobulin?

a. Anti-gamma-globulin antibodies; IgM.
b. Anti-gamma-globulin antibodies: IgG.
c. Anti-alpha-globulin antibodies; IgM.
d. Anti-alpha-globulin antibodies; IgG.

52. (433) Which one of the following statements concerning rheumatoid factors is true?

a. A negative test for rheumatoid factors rules out RA.
b. Eighty percent of actual cases have negative results.
c. The presence or absence of rheumatoid factors does not vary with the age of the patient.
d. Twenty percent of actual cases have negative results.

53. (434) In which one of the following tests for rheumatoid arthritis is a 1:20 dilution of patient's serum in glycine-saline buffer diluent mixed on a slide with a suspension of globulin-coated polystyrene latex particles?

a. Eosin-latex slide screening test.
b. Sensitized sheep cell slide test.
c. Latex slide screening test.
d. Latex macroscopic tube test.

54. (434) Polystyrene latex particles in RA tests are coated with

a. macroglobulin.
b. gamma globulin.
c. anti-gamma-globulin.
d. anti-rheumatoid-arthritis antibodies.

55. (435) Identify the latex agglutination test which uses latex particles coated with nucleoprotein.

a. Systemic lupus erythematosis (SLE).
b. C-reactive protein.
c. Gonorrhea.
d. Cryptococcosis.

56. (435) Which one of the following latex agglutination tests appears to be especially useful for detection of chronic cases, such as asymptomatic females?

a. Cryptococcosis.
b. Histoplasmosis.
c. Trichinosis.
d. Gonorrhea.

57. (436) Precipitation tests are usually performed by either capillary tubes or by

a. agglutination in agar.
b. neutralization in agar.
c. immunodifusion in agar.
d. hemagglutination-inhibition.

58. (436) In the capillary-tube precipitin test after incubation in an upright position, the precipitate usually forms at what part of the tube mixture?

a. In the antigen.
b. At the bottom.
c. In the antiserum.
d. At the antigen-antibody interface.

59. (437) Since the appearance of precipitation takes several hours, what technique is combined with diffusion in order to speed up the reaction?

a. Neutralization.
b. Agglutination.
c. Hemagglutination-inhibition.
d. Electrophoresis.

60. (437) In the single radial diffusion tests, where is the antigen placed?

a. Incorporated into the medium.
b. In a well.
c. On the ring of precipitate.
d. In a separate well from the antisera.

61. (437) What are the main advantages of IEOP over double diffusion tests?

a. Increased speed and the ability to detect very small quantities of antigen.
b. Increased speed and the ability to detect very large quantities of antigen.
c. Moderate speed and the ability to detect very small quantities of antigen.
d. Moderate speed and the ability to detect very large quantities of antigen.

62. (438) In the antistreptolysin test, a serial dilution of suspected serum is exposed to which of the following reagents?

a. A standardized suspension of 1 percent group O cells.
b. A standardized streptolysin O solution.
c. A standardized solution of antistreptolysin reagent.
d. A standardized solution of neutralized streptolysin O.

63. (439) In the test for streptococcal exoenzymes, agglutination of the erythrocytes indicates the presence of antibodies in the serum against all of the following streptococcal exoenzymes except

a. NADase.
b. DNase.
c. streptokinase.
d. hydrolase.

64. (440) Which of the following statements concerning primary syphilis is not true?

a. Diagnosis is accomplished mainly by a reactive serologic test.
b. A nonreactive test does not rule out the disease.
c. Inadequate treatment in the primary stage will mask symptoms and lead to the more serious secondary stage.
d. The serologic tests are nearly always reactive during this period.

65. (440) Which of the following is the most characteristic of the latent stage of syphilis?

a. Most symptoms are still present.
b. A nonreactive serologic test on serum.
c. A reactive serologic test on CSF.
d. A nonreactive serologic test on CSF.

66. (441) Venereal syphilis differs from endemic syphilis in geographical occurrence and what other characteristic?

a. Mode of transmission.
b. Antibody response.
c. Causative agent.
d. Organs infected.

67. (441) Yaws differs from syphilis by all of the following characteristics except

a. yaws is predominantly a childhood disease.
b. congenital transmission is rare.
c. yaws does not involve the viscera or central nervous system.
d. congenital transmission is common.

68 (442) The antigen used in the microflocculation tests such as the VDRL and USR tests are composed of all constituents except which one of the following?

a. Carbon particles.
b. Cholesterol.
c. Lecithin.
d. Cardiolipin.

69. (442) In addition to their usefulness as screening tests, nontreponemal tests serve what other purpose?

a. Detect biological false positives.
b. Follow the effectiveness of therapy.
c. Distinguish between the types of reaginic antibodies.
d. Distinguish between syphilis and nonvenereal syphilis.

70. (443) Reagin appears in the blood of a syphilitic patient about how many weeks after infection?

a. 1 to 2 weeks.
b. 2 to 3 weeks.
c. 4 to 5 weeks.
d. 6 to 8 weeks.

71. (444) In the RPR test, what quantity of unheated serum is spread within an 18 mm circle on a plastic card?

a. 0.5 ml.
b. 0.05 ml.
c. 0.15 ml.
d. 0.015 ml.

72. (444) In the RPR test, sera producing flocculation are reported in what manner?

a. Positive; titer not necessary.
b. Reactive along with a titer.
c. Weakly positive; titer not necessary.
d. Weakly reactive; titer not necessary.

73. (445) The VDRL slide test antigen for CSF is made sensitized for the test by adding an equal volume of

a. 1 percent saline.
b. 10 percent saline.
c. 20 percent saline.
d. 30 percent saline.

74. (445) In the latent stage of syphilis, the spinal fluid must be

a. reactive.
b. weakly positive.
c. positive.
d. nonreactive.

75. (445) The colloidal gold test on spinal fluid was widely used in the past for differentiation of the various types of

a. neurosyphilis and CNS conditions.
b. treponemes.
c. treponemal antibodies.
d. treponemal antigens.

76. (445) Which of the following reports is most commonly used for reporting the VDRL slide test results on spinal fluid?

a. Reactive or nonreactive.
b. Positive or negative.
c. Weakly reactive or nonreactive.
d. Weakly positive or negative.

77. (446) Identify the test which is severely limited in a laboratory because of a requirement for live treponemes from rabbits' testes?

a. Reiter Protein Complement Fixation (RPCF).
b. Automated Reagin test.
c. FTA-ABS (IgM).
d. Treponema Pallidum Immobilization (TPI) test.

78. (446) In which one of the following treponemal antigen tests have most recent applications involved the continuous filtering of the reactants on a moving strip of filter paper with visual interpretation of the flocculated carbon particles?

a. Treponemal Immobilization test.
b. FTA-ABS (IgM).
c. Automated Reagin test.
d. Rapid Reagin test.

79. (446) Which one of the following tests, considered sensitive and specific, is used to detect syphilis in babies?

a. FTA-CSF test.
b. Automated Reagin test.
c. FTA-ABS (IgM).
d. Reiter Protein Complement Fixation (RPCF).

80. (446) Which one of the following treponemal antigen tests usually remains reactive for life while most other serologic tests for syphilis become nonreactive, especially if treatment occurs before the tertiary stage?

a. Flourescent Treponemal Antibody Absorption (FTA-ABS) test.
b. Reiter Protein Complement Fixation (RPCF).
c. Automated Reagin test.
d. Treponema Pallidum Immobilization (TPI) test.

81. (447) Samples of glassware should be spot checked for residual detergent with which of the following indicators?

a. Methyl orange.
b. Brom cresol green.
c. Brom cresol purple.
d. Methyl red.

82. (447) Which one of the following conditions is least likely to contribute toward technician error?

a. Patients and visitors walking through the laboratory.
b. Strict attention to test performance.
c. Gossiping or miniskirts.
d. Loud radios.

83. (448) Vigorous mixing of sera so that foam occurs can

a. enhance antibody-antigen reaction.
b. enhance the sensitivity of the antigen.
c. cause hemolysis to occur.
d. cause denaturing of the antibodies.

84. (448) When using internal controls in serology, results must be

a. within the acceptable range previously established.
b. less than the acceptable range previously established.
c. above the acceptable range previously established.
d. within the acceptable range previously set by other serology laboratories.

85. (448) A proficiency testing program in serology allows comparison of results obtained from which one of the following sources?

a. Several different laboratories.
b. Within your own laboratory.
c. Several different manufacturers.
d. From the Epidemiological Laboratory only.

STUDENT REQUEST FOR ASSISTANCE

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AUTHORITY: 44 USC 3101. PRINCIPAL PURPOSE(S): To provide student assistance as requested by individual students. ROUTINE USES: This form is shipped with every ECI course package. It is utilized by the student, as needed, to place an inquiry with ECI. DISCLOSURE: Voluntary. The information requested on this form is needed for expeditious handling of the student's need. Failure to provide all information would result in slower action or inability to provide assistance.

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